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**A STUDY OF STOMATAL PATTERNS IN**  
*Plantago lanceolata L.*  
*Acer pseudoplatanus L.*  
*Sesleria caerulea L.*  
**IN RELATION TO ENVIRONMENTAL PARAMETERS**

**MSc. Thesis**

by

**RACHEL FERRIS**

**Being a dissertation submitted to the**  
**University of Durham**  
**as part of the degree of Master of Science 1991.**



**22 SEP 1992**

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### **List of Abbreviations**

ANOVA	Analysis of Variance Analysis
App(s)	Appendix/appendices
Chl	Chlorophyll
Chl a	Chl a
Chl b	Chl b
Chla/chlb	Chlorophyll a per chlorophyll b
cm <sup>2</sup>	Square centimetre
CO <sub>2</sub>	Carbon dioxide
DF	Degrees of Freedom
EIL	Electronic Instruments Limited
F	Variance Ratio
Fig.	Figure
Fr. wt.	Fresh Weight
g	Gram(s)
l	Litre
LMgraph	Light microscope photograph
m	Metre
mm <sup>2</sup>	Square Millimetre
MANOVA	Multivariate Analysis of Variance Analysis
PAR	Photosynthetically active radiation
P	Probability
PS	Photosynthetic/photosynthesis
r	Correlation coefficient
Scheffe	Scheffe Range Test
SD	Standard Deviation
SE	Standard Error of the mean
SEM	Scanning Electron Microscope/Micrograph
SI	Stomatal index/indices
SLA	Specific Leaf Area
V	Coefficient of Variation

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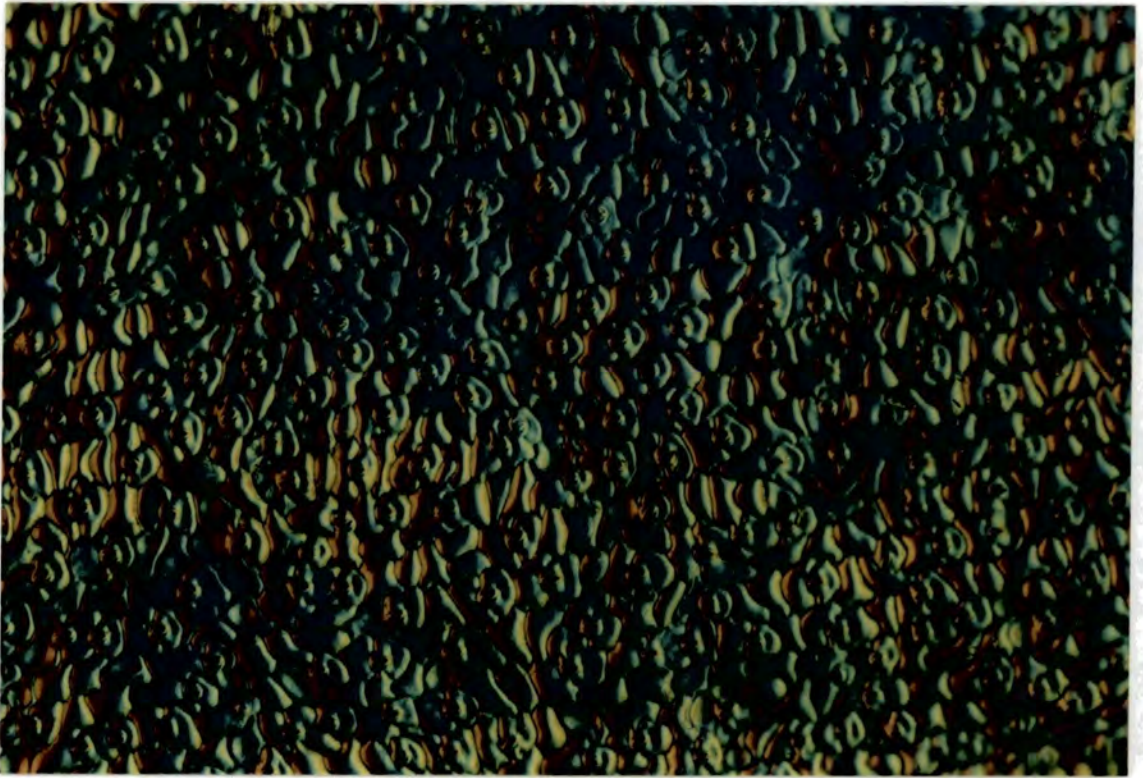


PLATE 1 A Light microscope colour photograph of the lower epidermis of *P. lanceolata* L. showing numerous stomata and epidermal cells. (x 100).

## **ABSTRACT**

A baseline study of leaf stomatal density, stomatal indices, guard cell length and leaf area was made in three species; a grass, *Sesleria caerulea* L., a herb, *Plantago lanceolata* L., and a tree, *Acer pseudoplatanus* L. in relation to environmental parameters. Chlorophyll analysis was also investigated to see if a relationship existed between density of stomata and chlorophyll content.

Multivariate analysis of variance showed: significant variation of the stomatal index (SI) and stomatal density in the three species in situ in the field: generally both parameters increasing with increasing altitude but with some variation across the *P. lanceolata* L. sites. Leaf area was significantly reduced at higher altitudes in all species. Guard cell length significantly decreased with increasing altitude in *S. caerulea* L., and *A. pseudoplatanus* L. but varied in *P. lanceolata* L. across sites.

Within habitat, *P. lanceolata* L. appeared to show no significant difference in the SI compared to between habitat variation at both sites studied. Stomatal density was generally greater on the abaxial leaf surface of *P. lanceolata* L. Apart from two plant populations, no significant difference was found in the SI between the adaxial and abaxial surface. Regression analysis showed soil depth, moisture and altitude explained a considerable amount of the variation in the data.

Transplants of *Acer pseudoplatanus* L., showed a significant increase in stomatal density and the SI with increasing altitude. Guard Cell length significantly decreased with increasing altitude.

Experimentally water-stressed plants showed variation. *Sesleria caerulea* L. and *Plantago lanceolata* L., both significantly increased stomatal density but decreased the SI with increasing stress. The exception was in the adaxial surface of the leaves of *Plantago lanceolata* L. from Widdybank Fell which showed no difference in the leaf SI between treatments. Leaf area was significantly reduced except in *S. caerulea* L. from Widdybank Fell. The SI and stomatal density increased with increasing water stress in *Acer pseudoplatanus* L. Guard cell length decreased significantly in the three species under water stress.

Shading of *Acer pseudoplatanus* L. significantly reduced stomatal density and the SI but increased the number of stomata per leaf. Leaf area and guard cell size significantly increased under shade.





Total chlorophyll (chl) significantly increased with increasing altitude in *S. caerulea* L. and *A. pseudoplatanus* L. but varied in *P. lanceolata* L. across sites. Total Chl decreased with increasing altitude in *A. pseudoplatanus* L. transplants. Full grown low light leaves of *A. pseudoplatanus* L. had more total chlorophyll per unit fresh weight than high-light leaves but the content per unit area of leaf surface was lower in low-light leaves. Under a varied watering regime, the two species studied for chlorophyll content (*A. pseudoplatanus* L. and *P. lanceolata* L.), showed a differential response, total chl increasing slightly in *A. pseudoplatanus* L. but with no pattern emerging in *P. lanceolata* L.

Conclusions drawn from this study indicate there is considerable variation both in the density and proportion of stomata produced (Stomatal index) in these three species both in the field and under manipulated environmental conditions which may have both a genetic and plastic base. This suggests care should be taken in interpreting morphological, anatomical and physiological changes in plants which may result from climatic change, in particular increased CO<sub>2</sub> levels.

#### **KEY WORDS:**

Stomatal Index, Stomatal density, leaf area, guard cell length, shading, water stress, altitude, soil depth and moisture

## CHAPTER 1

### INTRODUCTION

Considerable interest is being shown in the morphological and anatomical adaptations which may occur when plants are subjected to the rigours of environmental and climatic change. (Beerling & Chaloner, 1991, in press; Friend & Woodward, 1990; Norby & O'Neill 1989; and Pigott, 1974).

The epidermes of leaves of land plants are covered by a more or less impermeable outer layer, the cuticle. It restricts loss of water vapour but also entry of carbon dioxide (CO<sub>2</sub>) essential for photosynthesis. Stomata in the leaf epidermes enable gaseous exchange between the inside of the leaf and ambient air.

Stomata are the most important control system of CO<sub>2</sub> influx and water vapour efflux; the wider the stomata open the more CO<sub>2</sub> can be gained allowing photosynthetic reduction to take place but also more water vapour is lost via transpiration. By variations in the width of the stomatal pores, through changes in turgor pressure, these two contradictory processes can be optimized.

Much research effort has gone into investigating the physiology of stomata, see Cowan (1977), Heath & Meidner (1957), Jarvis & Mansfield (1981), Meidner & Mansfield (1968) and Willmer (1983). Less work has been undertaken on the underlying anatomical and developmental aspects of stomata in relation to varying environmental conditions, see Korner & Mayr (1981), Turner (1979), Woodward (1986) and Woodward & Bazzaz (1988). The early literature on stomata density and sizes has been reviewed by Ticha (1982).

Increased interest in the anatomy and development of stomata has been stimulated through a need to equate potential changes in plants with climatic change. In particular, attention is now being paid to the regulatory role of carbon dioxide, levels of which have risen from 300 ppm in 1900 to 350 ppm at the present time and are still increasing (Gribbon, 1986). The growth and development of plants affected by CO<sub>2</sub> and interspecific differences in these responses are well documented, see Dornhoff & Shibles (1976), Mott (1990), Sastek & Strain (1991), Strain (1987), Thomas & Harvey (1983), Woodward, Thompson & McKee (1991) and Wulff & Alexander (1985).

Examination of the plant's response not only to CO<sub>2</sub> levels but to a combination of environmental factors is essential before one can attribute changes in stomatal density in the field to CO<sub>2</sub> levels alone.

Recent studies have focussed on the stomatal density in fossil material (Beerling *et al.*, 1991; Penuelas & Matamala, 1990; Woodward 1987). Woodward (1987) has shown from herbarium leaves and by controlled experiments (Woodward & Bazzaz, 1988) at different CO<sub>2</sub> levels that stomatal density and index increase markedly as the CO<sub>2</sub> partial pressure is reduced below 34 Pa (or 340  $\mu\text{mol mol}^{-1}$ ). Stomatal density responded to reduced partial pressure of CO<sub>2</sub> in a simulation of high altitude (3,000m) when the CO<sub>2</sub> mole fraction was unchanged. At low saturation pressure deficits the increases in stomatal density cause increases in stomatal conductance but, in combination with a decrease in photosynthetic rate, this leads to a reduction in water use efficiency. Increasing CO<sub>2</sub> levels above 34 Pa appear to have limited effects on stomatal density (Thomas and Harvey 1983, Woodward 1986). A 40% decrease in the density of stomata was observed in herbarium specimens of *Acer pseudoplatanus* L., *Quercus robur* L., *Rumex crispus* L. and *Vaccinium myrtillus* L. collected over the last 200 years and this has been equated with increases in the partial pressure of CO<sub>2</sub> in the atmosphere. (Woodward, 1987).

Thus, leaves on many present day trees have far fewer stomata than their forebears. Reduction in stomatal numbers means that the unavoidable water losses from plants that accompany other gas exchanges are now inevitably lower than in the past, ie. they are now potentially more resistant to drought.

It is well known that stomata number and length vary among species (Boonkerd, 1987; Ciha & Brun, 1975; Cole & Dobrenz, 1970; Hirano, 1931; Meidner & Mansfield, 1968; Miskin & Rasmusson, 1970; Smith, Weyers & Berry, 1989; Teare, Peterson & Law, 1971). It also has been shown that there are differences in stomatal density on leaves of the same species which appear to depend upon the environment in which the leaves developed. Variation is also influenced by leaf growth, form, insertion and venation (Penfound 1931; Salisbury, 1928). Light intensity, water availability, temperature, CO<sub>2</sub> concentration and humidity have been known to affect stomatal density (Meidner & Mansfield 1968, Losch & Tenhunen, 1981 and Willmer, 1983). Early studies of many species growing in the tropics have shown they may have more than 500 stomata per square millimetre, whilst those outside have a lower density (Hirano, 1931).

Stomatal density may also tend to increase on leaves higher on the stem, lower leaves tend to have larger but less numerous stomata than those of higher insertion: this tendency is greater on the abaxial than adaxial leaf surface leading to a smaller adaxial:abaxial frequency ratio in leaves of higher insertion (Maeda, 1959). Stomatal characteristics of adaxial and abaxial

leaf surfaces can also differ markedly: the frequency of abaxial stomata usually exceeds that of adaxial stomata which are sometimes completely absent.

Stomatal density has been found to be inversely proportional to the area of the lamina and Gupta (1961) concluded the absolute stomata number to be constant within leaves of a single branch. Leaf area development is important: the growth of a leaf is the result of both cell division and cell expansion. It is the timing and magnitude of cell expansion which is important in determining final leaf size.

Esau (1977) describes stomatal differentiation as arising through differential divisions in the protoderm, which became secondarily meristematic, forming guard mother cells. The guard and cell mother cells, in turn, divide into the two guard cells. In order to take into account cell size in general Salisbury (1928) introduced a more stable characteristic of the epidermal stomatal complex which relates the number of stomata per unit area (S) to the number of epidermal cells per unit area (E) where Stomatal Index (SI) =  $[S/(E+S)] \times 100$ .

Earlier scientists found, by means of this index, that the proportion of stomata formed in the epidermis is no greater for sun leaves than for shade leaves. Similarly, the increased stomata densities in plants grown on dry soil as compared with those on wet soil or in small leaves as compared with large leaves are both due chiefly to differences in the growth of the epidermal cells, i.e. to differences in the spacing of the stomata and not to differences in the proportion of stomata developed. This appears to be true also for the variations in stomata density in different parts of the same leaf (Ticha, 1982). An early exception was the effect of high humidity which tends to reduce the proportion of stomata formed. Aquatic plants tend to have a low stomatal index (Salisbury, 1928).

Contrary to earlier opinion, however, the stomatal index is also known to be higher where conditions are more harsh (Jarvis & Mansfield 1981, Meidner & Mansfield 1968, Willmer 1983) and may vary for plants of the same species growing under different environmental conditions. At present the effect of environment is unresolved and may differ from species to species. Recent researchers have shown that the stomatal index of a dicotyledonous leaf varies with the light intensity received by the plant (Rahim & Fordham, 1990; Schoch, 1972, 1978, 1987; Schoch & Zinsou, 1975; Schoch, Lecharny and Zinsou, 1977; Schoch, Zinsou and Sibi, 1980 and Schurman, 1959) that the stomatal index of a dicotyledonous leaf varies with the light intensity received by the plant. A decrease in the stomatal index has also been found in wheat subjected to water stress (Gregory, 1991 unpublished).

It has been known for a long time that plants which grow in a sun or shade locality are adapted and acclimated to it and, consequently they differ in many anatomical and physiological characteristics. For recent reviews see Björkman (1981) and Boardman (1977). Shading increases leaf surface, cell division and expansion and decreases the number of stomata per mm<sup>2</sup> and the percentage of stomata in relation to other cells in pepper (*Capsicum annuum* L.), (Schoch, 1972). In contrast, Rahim and Fordham (1991) reported in the garlic cultivar 'Bangladesh' and 'Fructidor' that the stomatal index decreased with increasing light intensity and leaf thickness increased with light intensity with gains in leaf dry weight per unit area. An increase in the light intensity reduced leaf length and size of epidermal cells. The literature in this field is unresolved.

Moisture level and soil depth can drastically affect stomata density and the multiplication and expansion of cells, (Rawson & Craven, 1980; Seyed-Yagoobi, 1977; Terry, Waldron & Olrich, 1971 and Yegappan *et al.*, 1982. Fewer stomata per unit area were present in plants growing under 'optimum' soil moisture conditions (Penfound, 1931). Water stress can increase stomata density, but reduce stomata sizes and area so that the area of the stomata apparatus per unit leaf area or the number of stomata per leaf remains unchanged. A re-examination using the stomatal index as the parameter is important.

The influence of altitude on plant growth and distribution has concerned plant ecologists for a long time (see Tranquillini, 1979 for reviews). Temperature and atmospheric pressure decrease with altitude whilst wind speed increases. Irradiance may increase with altitude on clear days, although in the long term mean irradiance may decrease, dependent on the frequency and depth of cloud cover. General altitudinal variations of climate in mountain regions cause many visible changes in the distribution and composition of the vegetation, the growth habit, and the anatomy and physiology of individual plants, see Korner, Bannister and Mark (1986), Korner & Cochrane (1985), and Woodward (1973, 1975, 1983, 1986). Altitudinal effects on photosynthetic CO<sub>2</sub> uptake in plants have been examined by Smith and Donahue (1991) in relation to changes in leaf and air temperature and CO<sub>2</sub> concentration and ambient pressure. In *Vaccinium myrtillus* L. growing under saturating irradiance, optimum temperatures and a range of vapour pressure deficits, photosynthetic rate and stomatal conductance increased with the altitude of origin of the populations. Correlated with these increases was an increase in the adaxial stomatal density with altitude, Woodward, (1986). In a study of alpine plant communities of the Austrian central Alps, Korner & Mayr (1981), showed that 70% of the species investigated were amphistomatous, with stomatal frequency increasing with altitude.

It has been shown that plants living at high altitudes show, due to a better supply of radiant energy, decreased chlorophyll (chl) and carotenoid contents per fresh matter, especially in the species with higher pigment concentrations, increased chl a/b ratio and a better photosynthetic efficiency (Todaria *et al.*, 1980).

Chlorophyll content of photosynthetic organs varies according to the plant species, leaf position, plant age and growth phase, grade of ecotypic adaptation and the environmental conditions in particular irradiance, temperature, water stress and nutrition. It is not only the total chlorophyll content that is important but the amount of chl a:chl b, as the ratio of these is thought to be an important adaptation to light intensity and temperature (Mooney & Billings 1961, Tieszen & Johnson, 1968). However, there seems to be a homeostatic mechanism in natural communities which keeps chlorophyll content within the range 0.1 to 3.0 g m<sup>-2</sup> (Odum *et al.*, 1958b).

The study reported here aimed to examine a number of selected species: a grass, *Sesleria caerulea* L., a herb, *Plantago lanceolata* L. and a tree species, *Acer pseudoplatanus* L. to determine the baseline variations in stomatal parameters in situ in the field in relation to variations in habitat both within and along altitudinal gradients. In addition experimental manipulation of the growing environment was undertaken to assess effects on development. Parameters studied included stomatal density per mm<sup>2</sup>, stomatal index and guard cell length; chlorophyll levels and leaf area.

The study of the three chosen species also involved:

- 1) Transplant experiments, in order to establish the degree of plant plasticity in response to the environment;
- 2) Controlled experiments: effects of shading and water stress (different watering regimes) on stomatal parameters, leaf area and chlorophyll levels;
- 3) Chlorophyll analysis was carried out on field material to determine if a relationship existed between stomatal density and the level of chlorophyll in the leaf over an altitudinal range; and on shaded and water stressed plants to see if different light or watering regimes affected the levels.

This study proposed, in particular, to test the hypothesis that there is no difference in the stomatal index with altitude or under the influence of changing environmental conditions as the plants are able to maintain a stable index through differential growth of the epidermal cells.

Techniques used included light and electron microscopy, planimetry, and measurements of cell dimensions and frequency using stereological methods and chlorophyll extraction.

The overall aim of the project was to obtain a baseline measure of stomatal parameters in relation to habitat and environmental variation in the three chosen species from Northern England. Once a contemporary pattern has emerged one can put into context changes which may result from climatic change.

## CHAPTER 2

### MATERIALS AND METHODS

#### 2.1 Plant Materials

A grass, *Sesleria caerulea* L., (Blue Moor Grass), a herb, *Plantago lanceolata* L. (Ribwort Plantain) and a tree species, *Acer pseudoplatanus* L. (Sycamore) were collected from a variety of sites, which encompassed lowland, upland, and coastal regions in the North of England. (Fig 1). This was in order to cover a variety of different environmental habitats and altitudinal ranges. The species collected or transplanted at each site are indicated against the site descriptions below. In text, species are referred as *S. caerulea* L., *P. lanceolata* L. and *A. pseudoplatanus* L.

The distribution and characteristics of the three species chosen in this study are indicated below.

*Sesleria caerulea* L. (Blue Moor Grass), a graminea, which exists as several edaphic and climatic ecotypes (Round & Turner 1968). The species is found mainly in open habitats on basic rich soils in Northern England, Western Ireland and Scotland. It has a wide tolerance of various soil-water conditions. The leaves are keeled, glaucous and dark-green beneath. The panicle, borne in May-June is blue-grey and glistening.

*Plantago lanceolata* L. (Ribwort Plantain) from the Plantaginacea family is common throughout the British Isles. It occurs on grassland on moist-dry, and acid-calcareous ground. It has a wide altitudinal range and is a perennial. Leaves are long, narrow and strongly ribbed.

*Acer pseudoplatanus* L. (Sycamore) from the family Aceracea, is a native tree of the Central and Northern European mountains and was introduced into Britain in the 15th and 16th Centuries. It occurs in exposed, coastal areas, on high limestone hills and in city environments. Sycamore prefers deep, moist, well-drained soils and is tolerant of exposure and salt spray. The leaves are 5-lobed, dark green above and glaucous beneath, ovate and irregularly toothed.

Climatic data from Widdybank Fell Weather Station, Durham Observatory and Sunderland Polytechnic show the variation in climate between coastal, inland and upland sites during the period of the experiment. (See App. 3).

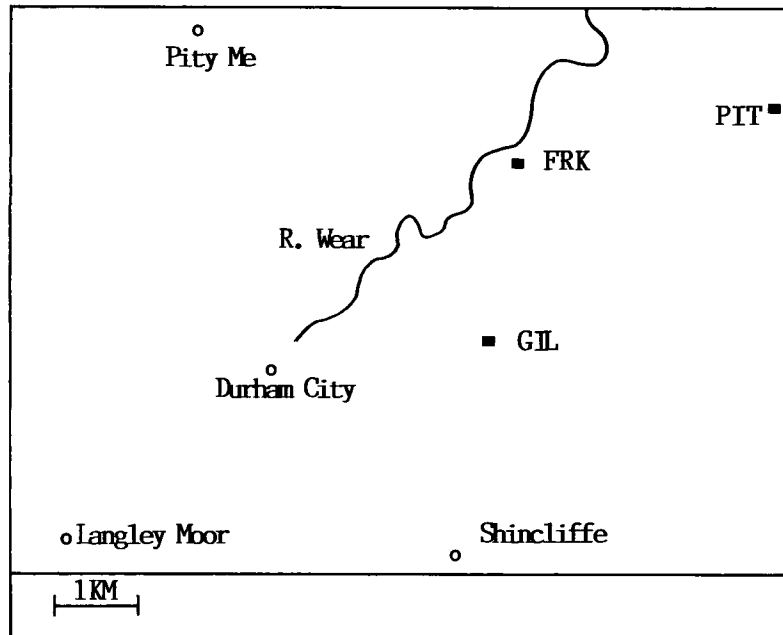
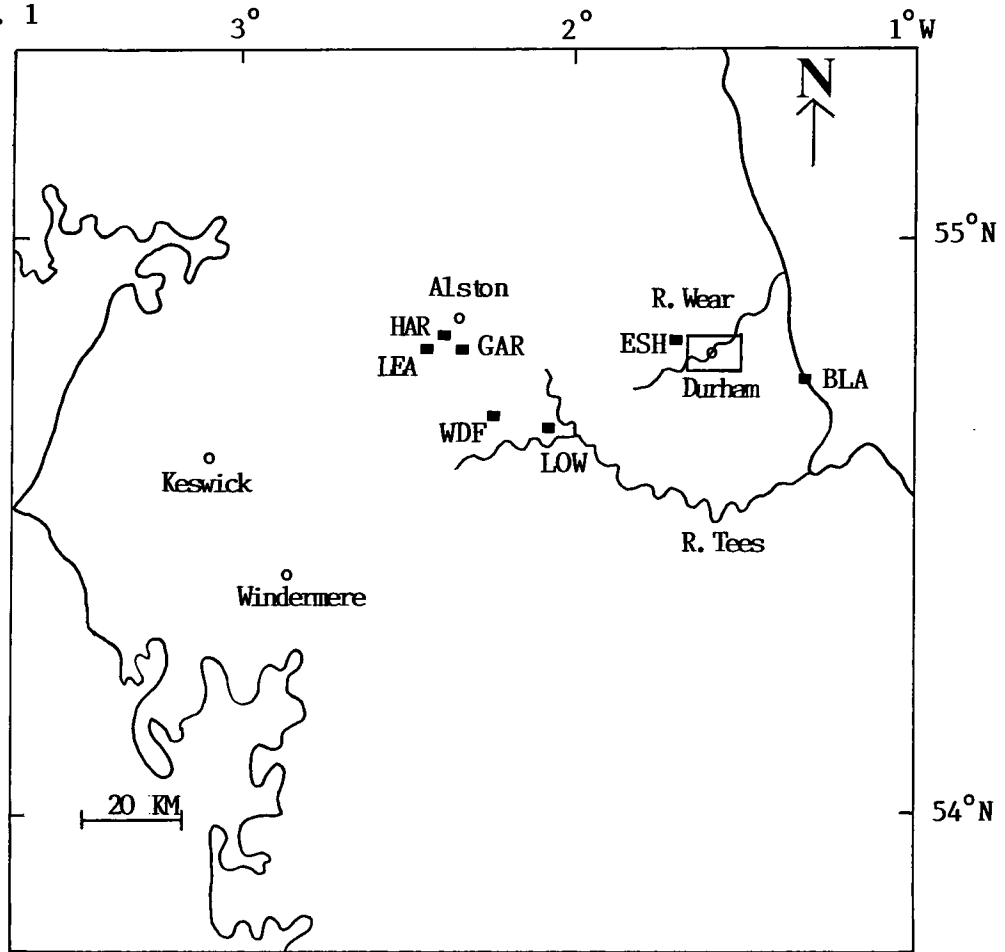


**Fig. 1**                      A Map of part of North East England showing the locations of ten selected sites chosen for plant collection and/or transplant experiments in this study. Sites close to Durham are shown in the bottom diagram.

**KEY:**                      ■       Selected site

HAR	=	Hartside Nursery, Alston, Cumbria. 350m
ESH	=	Esh, Durham. 210m
WDF	=	Widdybank Fell, Upper Teesdale. 529m
GAR	=	Garrigil, Cumbria. 340m
LEA	=	Leadgate, Cumbria. 350m
PIT	=	Pittington Hill, Co Durham. 120-150m
LOW	=	Low Force, Teesdale. 275m
BLA	=	Blackhall Rocks, Blackhall. 10m
GIL	=	Gilesgate Moor, Durham. 83m
FRK	=	Frankland & Kepier Wood, Durham. 35-65m

FIG. 1



## 2.2 Description of Sites

### SITE 1. Blackhall Rocks, Co. Durham (NZ468395)

This is an exposed coastal site about 10m above sea level facing east. It has deep, flushed soil on the cliff top (pH 7.8) over magnesium limestone. *S. caerulea* L. and *P. lanceolata* L. were collected. (Plate 2).

### SITE 2. Frankland and Kepier woods, Co. Durham (NZ297449)

An 80 hectare inland woodland site, between 35-60m above sea-level and occupying a steep aspect on the banks of the River Wear. It has a moderately deep brown earth soil (pH 5.9), with unstable sandstone cliffs. *A. pseudoplatanus* L. leaves were collected.

### SITE 3. Gilesgate Moor, Co. Durham (NZ295434)

An inland, sheltered site, which is a private garden, about 83m above sea-level. It has a shallow, brown earth soil (pH 6.5). This site was the source of the Sycamore seedlings, all from the same province, used in the Field Transplant and Shading experiments.

### SITE 4. Esh, Co. Durham (NZ198442)

This site is situated on a hill top about 210m above sea-level and about 8 km from Durham city. The site is open and exposed. *A. pseudoplatanus* L. seedlings were transplanted out at this site.

### SITE 5. Pittington Hill, Co. Durham (NZ331447)

This is a 6.4 hectare area of magnesium limestone grassland, which is partly grazed, and steep in places, facing west. The site is between 120-150m above sea-level. The exposed area has very shallow soil, but deeper soil occurs in the few sheltered areas, with clay or till overlaying the limestone. Soil pH is 7.9 - 8.0. *P. lanceolata* L. plants were collected from both exposed and shaded habitats, and *S. caerulea* L. only from the exposed plateau. (Plate 3).

### SITE 6. Low Force (NY903279)

An inland site on the Whinsill, on the banks of the River Tees, and approximately 275m above sea-level. Plants of *S. caerulea* L. were collected from a rock outcrop. *P. lanceolata* L. plants were collected on an island further upstream where the soil was deeper (pH 7.4). Leaves of *A. pseudoplatanus* L. were collected from trees downstream occupying damp soil (pH 6.9). (Plate 4).



PLATE 2 Study Site at Blackhall Rocks



PLATE 3 Study Site at Pittington Hill





PLATE 4 Study Site at Low Force, Teesdale



PLATE 5 Study Site at Widdybank Fell, Upper Teesdale

SITE 7. Hartside Nursery, Alston, Cumbria. (NY707446)

This inland site is very open and exposed and faces south east. The site stands 330m above sea-level. The soil is a brown earth with a pH of 6.5. *A. pseudoplatanus* L. seedlings were transplanted at this site and leaves of the same species were collected.

SITE 8. Leadgate, Nr Alston, Cumbria. (N707438)

An inland exposed roadside verge which is dominated by *P. lanceolata* L., about 340m above sea-level. It has a shallow, peaty, stony soil (pH 6.6). *P. lanceolata* L. plants were collected.

SITE 9. Garrigil, Nr Alston, Cumbria. (NZ745414)

An inland, exposed site close to the River South Tyne, about 330m above sea-level. *P. lanceolata* L. was collected from two habitats: a population with small leaves occupying shallow, stony ground close to the river, and also from a population with larger leaves and growing in deeper, peaty soil. Soil pH was 7.1.

SITE 10. Widdybank Fell, Upper Teesdale. (NY817297)

An inland site on the Pennines about 500m above sea-level. The area is exposed open moorland, with a shallow layer of soil (pH 7.6) over carboniferous limestone. The site is fairly well drained. *P. lanceolata* L. and *S. caerulea* L. plants were collected. (Plate 5).

## **2.3 FIELD DATA**

### **Plant Collection**

Plants of *S. caerulea* L., *P. lanceolata* L. and *A. pseudoplatanus* L. seedlings together with leaves of the latter species were collected from the study sites indicated during May, June and July 1991. (See Plates 6, 7 and 8).

The plants were potted up in a Levington Compost mixture in 12 x 15cm pots (Sycamore) and 10 x 7cm pots (Blue Moor grass and Ribwort Plantain) and kept in the laboratory while they established themselves. After a few days they were transferred to a plant growth room for use in experimental work. The plants were watered as required. Samples of leaves from the plants were frozen for use in chlorophyll extraction.





PLATE 6 *S. caerulea* L. growing out of a rock at Low Force,  
Teesdale



PLATE 7 *P. lanceolata* L. growing in shaded habitat at  
Pittington Hill, Co. Durham





PLATE 8 Two *A. pseudoplatanus* L. seedlings from Gilesgate Moor, Co. Durham. The seedling on the left attained its size after 20 days of growing in the pot. The young seedling on the right is becoming established.



PLATE 9 *P. lanceolata* L. and *S. caerulea* L. from both Blackhall Rocks and Widdybank Fell growing in pots in the plant growth room. The plants are under varying methods of watering.



## 2.4 Soil pH

The soil pH of each site was measured using an EIL pH meter (7020).

- (1) Approximately 20g of fresh, moist soil was weighed, and
- (2) added to 25ml of distilled water, stirred and allowed to stand for ten minutes.
- (3) The pH was recorded when stable. A mean reading from three samples per site was taken.

## 2.5 Soil Moisture Content

A standard gravimetric method was used which measured the loss in mass when a moist soil was dried in an oven at 105°C overnight.

The air dry and field moisture content of the soils at each site was determined (calculation 1), and the percentage moisture obtained on an oven dry basis when the soils were at field capacity (Calculation 2). Both methods were applied. However, results using Calculation 1 have been presented in this study.

- (1) A porcelain basin or tin was weighed accurately and its mass (Ma) recorded.
- (2) Approximately 10g (or 20g if available) of soil sample was added (repeats were made with soils at field capacity) and reweighed accurately to give Mb, and
- (3) placed in an oven overnight at 105°C and cooled in a desiccator and weighed accurately to give Mc.

### Calculation 1:

$$\frac{\text{Mass of Sample taken} - \text{Mass of oven dry sample}}{\text{Mass of sample taken}}$$

(air dry or field moist basis). Avery *et al.*, (1974)

### Calculation 2

$$\text{Moisture Content} = \frac{\text{Mb} - \text{Mc}}{\text{Mc} - \text{Ma}} \times 100 \%$$

McRae (1988)

## 2.6 Stomatal Counts and Guard Cell Measurements

Two methods were used in this study to obtain stomatal/epidermal cell counts. Leaves of similar size, for each species were gently washed in a 1% solution of Teepol.

1) Epidermal Peels: When dry, a thin layer of clear nail varnish was painted onto the abaxial surface of each leaf in *A. pseudoplatanus* L., adaxial in *S. caerulea* L. and both abaxial and adaxial in *P. lanceolata* L.). The part of the leaf used in this study was midway between the tip and the base (Fig. 2a, b & c), for each species. In this study five leaves (each from an individual plant) were used. Areas in the vicinity of large veins and trichomes were avoided.

The layers were allowed to dry before being gently peeled off with tweezers and placed on a microscope slide, and covered with a cover slip. The slides were examined under the light microscope to obtain the stomatal density and the stomatal indices of the leaves. The number of stomata and the number of other epidermal cells were counted from five whole fields of view (area 0.09775 mm<sup>2</sup>) from each of five individual leaf surface peels (total 25 fields) at x400 magnification. In the case of sycamore five half fields of view (area 0.048875 mm<sup>2</sup>) were examined. The number of stomata were converted to number of stomata/mm<sup>2</sup>. From the data the 'stomatal index' was calculated which relates the number of stomata per unit area (S) to the number of epidermal cells per unit area (E) where the Stomatal Index (SI) =  $[S/(E+S)] \times 100$ . (Salisbury, 1928).

Guard cell lengths were measured under the same magnifications from five fields of view on each of five leaves giving a mean length from 25 measurements. A calibrated microscope slide was used to convert measurements to micrometres.

### 2) Ethyl Acetate Impressions

Clear Acetate sheets were cut into small squares. The leaf section to be studied was placed on the acetate sheet, either abaxial or adaxial side down depending on the species. A few drops of ethyl acetate was placed between the leaf and the acetate. The leaf section was heavily weighted for about two minutes, and then gently peeled away, leaving an impression on the acetate. In order to secure the leaf impression for viewing and so to avoid distortion in magnification, the area of the leaf to be studied was cut out from the leaf impression and clamped between two slides or taped down with strong sticky tape.

This latter method was only used for some of the sycamore specimens from the field that failed to make good epidermal peels.

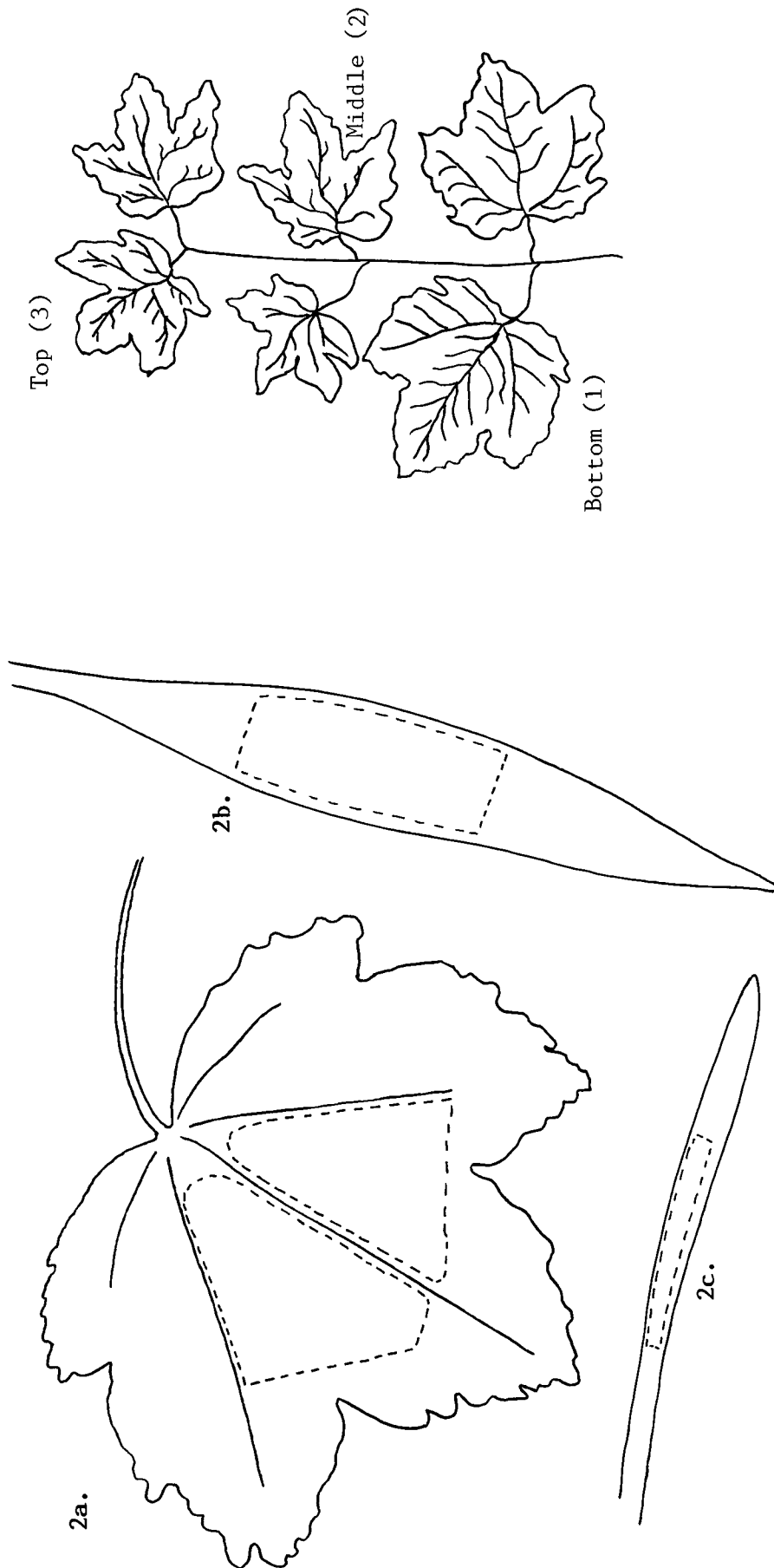


FIG. 2 Diagram illustrating the region of the leaf from which sections were taken (dotted line). 2a. *A. pseudoplatanus* L.; 2b. *P. lanceolata* L.; 2c. *S. caerulea* L.

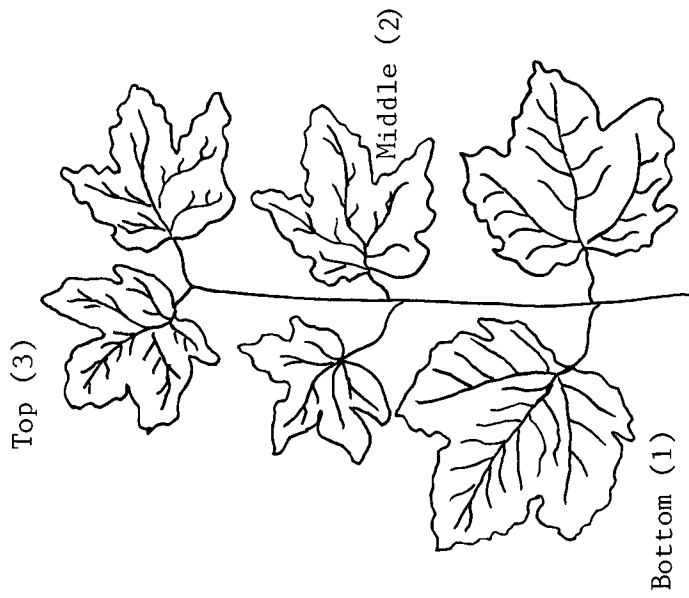


FIG. 3 Diagram illustrating the leaves used (1, 2, 3,) to study stomatal parameters within young Sycamore seedlings.

## 2.7 Leaf Area Measurements

Leaves of *P. lanceolata* L. and *A. pseudoplatanus* L. were photocopied to obtain an outline. The area within the outline was measured using a hand planimeter (Paquin and Coulombie 1959). Planimetry was repeated two times (with the arms of the instrument in different positions, and the means of the values indicated by the counter was then recorded. The leaf area of *S. caerulea* L. was the product of length x breadth measurements.

## 2.8 Transplants in the Field

Thirty *A. pseudoplatanus* L. seedlings collected in June from under one solitary tree at Site 3, Gillesgate Moor, County Durham, were potted up in 12 x 15cm pots in a Levington compost mixture and left for approximately ten days to establish in an unheated greenhouse. The seedlings were put out in their pots at three sites of different altitude at the end of June and left in situ for twenty days. The number of leaves on each seedling were tagged and noted before the transplant. Five plants were selected from these and leaves which had grown during the twenty days were used. Stomatal counts, the stomatal index, chlorophyll levels and leaf areas were obtained.

## 2.9 Experimental Work

### 2.9.1 Seedling Variation

Stomatal densities and stomatal indices were obtained from successive leaves up the stem of four young sycamore seedlings. Only three pairs of leaves were present (Fig. 3) and leaf samples were examined alternately. Guard cell length was measured in one plant only.

### 2.9.2 Effect of Shading

*A. pseudoplatanus* L. seedlings were collected from the same site as the Transplant seedlings and potted in 12 x 15cm pots containing a Levington Compost mixture and left for ten days to become established. A random block design was used, consisting of three treatments of six plants each, giving a total of eighteen samples (Fig. 4). A shading frame was constructed, consisting of 18, 10" x 13" grid squares of different relative shade intensities provided by Monolette netting of different light transmission values: 60% shade and 40% shade, together with a very fine white stockinette netting which cut out some incident light and acted as a control (almost 100% light transmission). A 16-hour photoperiod was operating and the plants were raised close to the netting (Plate 10). A Skye PAR Light meter was used to measure the relative



PLATE 10 *A. pseudoplatanus* L. seedlings under shading frame in growth room

60	40	60	40	60	40
100	60	100	60	100	60
40	100	40	100	40	100

FIG. 4 Diagram of Shading Frame based on a Random Block design.  
Relative shade intensities: 60% shade; 40% shade;  
Control - almost 100% of light in growth room.

light intensity which in the growth room was lower than normal daylight. A mean of six readings for each shading treatment was taken close to the plant and the relative intensities obtained.

	( $\times 10 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ )	670/630 nm
60% shade	- 25.65 $\mu\text{mol m}^{-2} \text{ s}^{-1}$	4.8
40% shade	- 51.80 $\mu\text{mol m}^{-2} \text{ s}^{-1}$	4.7
Control (almost 100% light of light in growth room)	- 90.10 $\mu\text{mol m}^{-2} \text{ s}^{-1}$	4.7

The relative humidity in the growth room was between 64% - 66% and the temperature was 24° - 25°C throughout the experimental period. The plants were watered daily to maintain soil conditions near field capacity. Leaves present at the start of treatment were marked with a permanent marker or tagged. Five weeks after the start of treatment, new, and where possible, fully expanded leaves were sampled at random from five plants within each treatment.

Five randomly chosen microscopic fields/leaf surface on five plants per treatment were examined and the mean stomatal density, stomatal index and guard cell lengths obtained. In addition the number of stomata/leaf surface was calculated from the stomatal density and leaf area measurements. Chlorophyll levels were also determined (see following page).

### 2.9.3 Effect of Water Stress

Plants from two populations of *S. caerulea* L. and *P. lanceolata* L. collected from both Blackhall Rocks and Widdybank Fell were potted in 10cm x 7cm pots containing a Levington Compost mixture, and placed in a controlled plant growth room (Plate 9). Seedlings of *Acer pseudoplatanus* L. were potted in larger pots (12 x 15cm) and placed in an unheated greenhouse. After an establishment period of five days, three regimes of water treatment were applied to each species for twenty days:

Watered every day (Control)

Watered every 4 days

Watered every 7 days

In the results section the terms 4-day and 7-day water stress apply to the above regimes. All treatments were arranged in a blocks design and replicated five times. The temperature in the growth room was between 24°C - 25°C and the humidity between 64% - 70% throughout the

duration of the experiment. Young expanded leaves were selected twenty days after the start of treatment. Leaf areas, stomatal counts, guard cell measurements and chlorophyll analysis of these plants were obtained following treatment.

## 2.10 Chlorophyll content

Chlorophyll levels were obtained for all three species at each site in the field, and also under experimental conditions, apart from *S. caerulea* L. plants under water stress, which produced insufficient new leaf material.

### Extraction

Leaves of similar age and stage of development were taken from five plants. The youngest new leaves were taken from those under water stress or under shade treatments. Depending on the quantity of leaf material available the following method was applied:

- 1) leaf samples of either 0.5g or 0.25g fresh weight were used.
- 2) the leaves were coarsely chopped; and
- 3) added to 10 ml or 6 ml respectively of 80% acetone, and left in the dark for 24 hours.

### Estimation

The optical density of the resulting solutions obtained was measured at 645 and 663 nm using a Pye Unicam Pu 8600 UV/VIS spectrophotometer. The readings of optical density (OD) from the spectrophotometer at wavelengths 654 and 663nm were used to calculate the total chlorophyll (tot chl), chlorophyll a (chl a) and chlorophyll b (chl b) contents using the equation:-

$$\begin{aligned} \text{total chlorophyll mg/l} &= 20.2 \times \text{OD}_{645} + 8.02 \times \text{OD}_{663} \\ \text{chlorophyll a} &= 12.7 \times \text{OD}_{663} + 2.69 \times \text{OD}_{645} \\ \text{chlorophyll b} &= 22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663} \end{aligned}$$

The results obtained were converted to chlorophyll/fresh weight for each species and the chlorophyll a/chlorophyll b ratio was calculated

## 2.11 Statistical analysis of data

Statistical analyses applied to the data in this study determined if there was a significant difference in the parameters measured between the plants from different populations, environmental habitats and altitudes. MANOVA, ANOVA and ONE-WAY analysis of variance was used, the latter with a Scheffe range test which determined where the differences occurred. (See Sokal & Rohlf, 1981 and SPSSX User Guide, 1988)

T-tests were employed to determine any difference in the Stomatal Index and Stomatal numbers between the upper and lower leaf surface in *P. lanceolata* L. Stepwise Multiple Regressions were performed on the field dependent variables against some environmental variables. F tables and T tables were used to find the significance of the results. These are shown in tables as:

\*\*\*\*  $P \leq 0.0001$

\*\*\*  $P \leq 0.001$

\*\*  $P \leq 0.01$

\*  $P \leq 0.05$

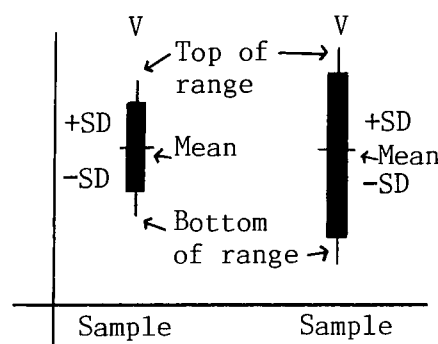
NS  $P > 0.05$  Not significant

Canonical Correspondence Analysis was also used but due to multicollinearity occurring, interpretation of the output was unsatisfactory.

SPSSX, a statistical package was used to analyse the data and Quatro, a spreadsheet package was used for data management and to aid in calculations.

The graphical data in this study show the arithmetic mean and standard error (SE) of 25 field of view counts unless otherwise stated. Graphs of guard cell length show the Arithmetic Mean, Standard Deviation (SD), Range and Coefficient of Variation (V) for 25 measurements. Fig 5. shows an example of one type of graph used in this study. All raw data is available from the author.

**Fig 5.**



The Coefficient of Variation (V) =

$$\frac{SD}{MEAN} \times 100$$



## 2.12 Photography

SEM: Leaf sections from each species were prepared for examination under the Scanning Electron Microscope using the method of A Boyde (1972). Small sections of tissue were mounted on 12 mm diameter pin type aluminium stubs, with double-sided sellotape and placed in liquid nitrogen and freeze-dried at  $-20^{\circ}\text{C}$  -  $-30^{\circ}\text{C}$ , in an Edwards tissue dryer for one to one and a half hours. Specimens were sputter coated under vacuum with gold paladium in an Argon atmosphere for three minutes (20 mA, 13 Pa, 37 mm working distance), and images were recorded on the SEM screen.

Light Microscopy: General characteristics of the stomata and epidermal cells of each species were photographed from the slides of acetate peels using a photomicroscope. Field photographs were taken with a Minolta X-300 camera.

Fluorescent Microscopy: The fluorescent microscope illuminates the specimen from above with the help of its objective lens. Sections of leaf were deftly cut with a sharp razor, and mounted on a slide in a solution of flurochrome, either calcofluor (binds to cellulose) or Auramino O (binds to lignin and cutin). This is a fast and straightforward method (see Gates, 1991).

## CHAPTER 3

### RESULTS

Summary results of stomatal parameters showing means and standard errors are shown in Appendix 1. Results of output from MANOVA, ANOVA, One-way ANOVA Scheffe Range Tests (Scheffe), T-tests and Stepwise Multiple Regressions are summarised in App. 2. These were the statistical tests used unless otherwise stated.

### 3.1 Field Studies

#### 3.1.1 In Situ

##### A. *S. caerulea* L.

Table 1 shows leaves of *S. caerulea* L. were larger at the lower altitudinal sites. The difference in leaf area was highly significant between sites ( $P < 0.0001$ ). Leaves from Pittington Hill were significantly different in size ( $P < 0.05$ ) from leaves collected from Blackhall Rocks, Low Force and Widdybank Fell (Scheffe). Plants at the upland site, Widdybank Fell, were noted to be smaller in size, with a mean leaf area of  $0.87 \text{ cm}^2$ .

**TABLE 1** Mean Leaf Area of populations of *S. caerulea* L. from four study sites of different altitude.

SITE	Altitude (m)	Area ( $\text{cm}^2$ ) $\pm$ SE
Blackhall Rocks	10	$1.28 \pm 0.16$
Pittington E	120-150	$3.38 \pm 0.61$
Low Force	275	$0.96 \pm 0.12$
Widdybank Fell	529	$0.87 \pm 0.09$
n = 5		****

Fig. 6 shows the Mean leaf Stomatal Index (SI) in leaves of *S. caerulea* L. from four field populations at sites of increasing altitude. A general decrease in the SI is observed up to 275m (Low Force) followed by an increase at 529m (Widdybank Fell) which was highly significant ( $P < 0.0001$ ) between sites. Differences in the SI lay between the Blackhall Rocks population and the other three sites; Pittington and Widdybank Fell were significantly different ( $P < 0.05$ ) from Low Force (Scheffe).

Fig. 7 shows the variation in leaf stomatal density in *S. caerulea* L. from four field populations, at sites of increasing altitude, the differences being highly significant ( $P < 0.0001$ ) between sites. Mean leaf stomatal density at the Blackhall coastal population is small ( $107.2 \pm$

**FIG. 6**      Mean Stomatal Index in leaves of  
*S. caerulea* L. collected from four  
populations over a wide altitudinal range.  
( $P < 0.0001$ ) ANOVA.

**FIG. 7**      Mean Stomatal Numbers/mm<sup>2</sup> in leaves of  
*S. caerulea* L. collected from four  
populations over a wide altitudinal range.  
( $P < 0.0001$ ) ANOVA

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 fields of view.

SITE:            Blackhall Rocks (10m)  
                  Pittington Hill (120-150m)  
                  Low Force (275m)  
                  Widdybank Fell (529m)

FIG. 6

## STOMATAL INDICES SESLERIA CAERULEA L.

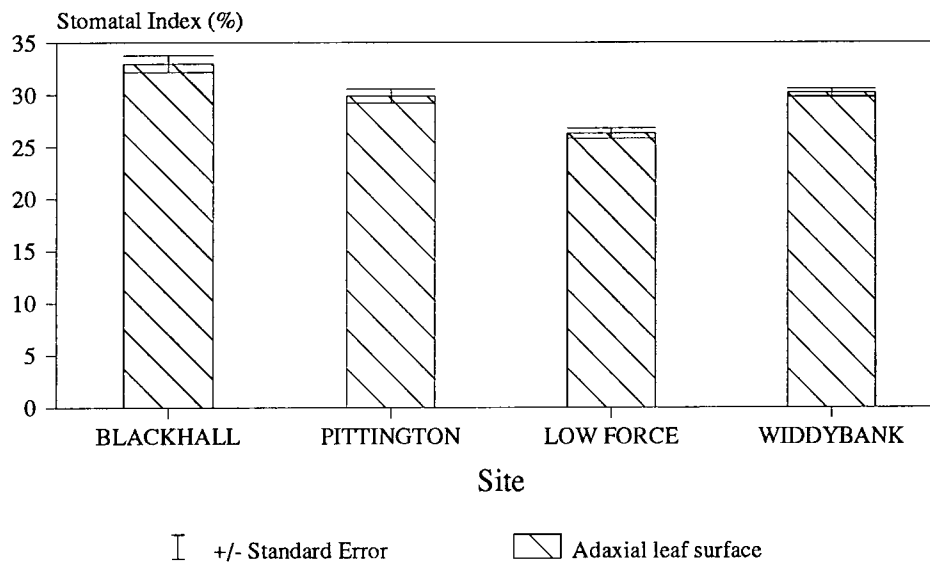
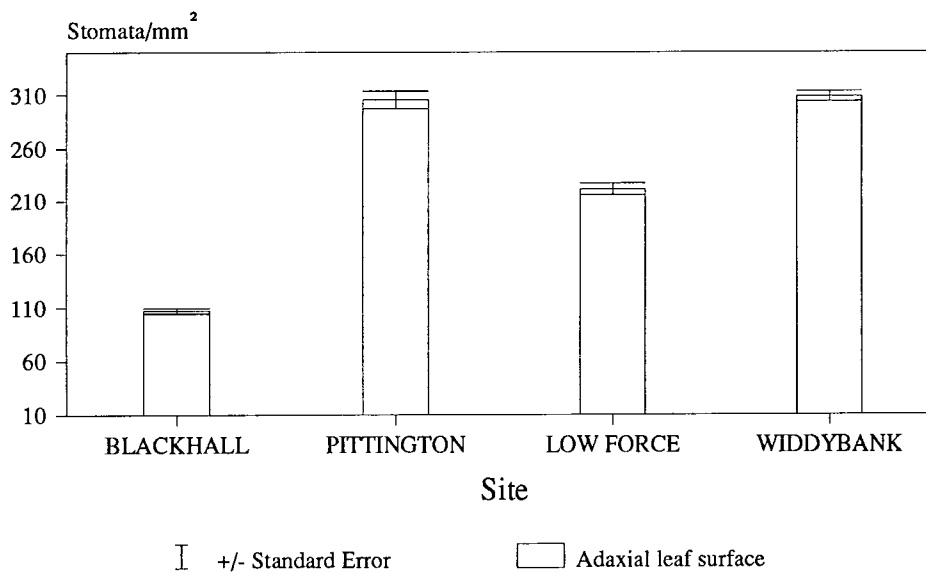


FIG. 7

## STOMATAL NUMBERS SESLERIA CAERULEA L.



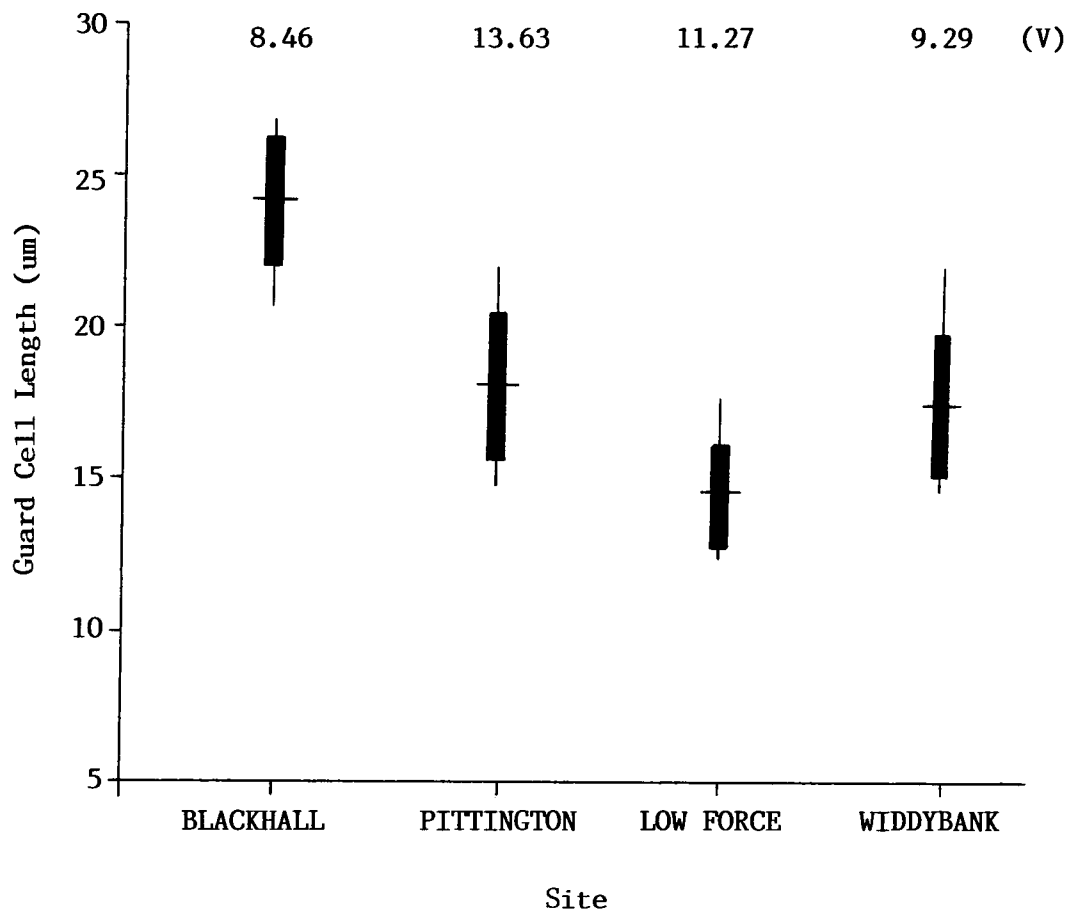
**FIG. 8**      Mean Guard Cell Length in leaves of  
*Sesleria caerulea* L. collected from four  
populations over a wide altitudinal range.  
( $P < 0.0001$ ) ANOVA.

The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

SITE:            Blackhall Rocks (10m)  
                  Pittington Hill (120-150m)  
                  Low Force (275m)  
                  Widdybank Fell (529m)

FIG. 8

## GUARD CELL LENGTH

*Sesleria caerulea* L.

2.6/mm<sup>2</sup>) and differs from Low Force ( $221 \pm 5.7/\text{mm}^2$ ). Pittington Hill and Widdybank Fell populations have higher and similar leaf stomatal densities ( $305.3 \pm 8.1/\text{mm}^2$  and  $308.5 \pm 4.7/\text{mm}^2$  respectively); both differing from Blackhall Rocks and Low Force at the 5% level of probability (Scheffe). See App. 2.

Leaf Guard cell length is shown to decrease significantly ( $P < 0.001$ ) with altitude up to 275m (Fig. 8) and is followed by a slight increase at the Widdybank Fell site (529m). The Range of lengths is small in the Low Force population and the Coefficient of Variation or spread in size is lower at Blackhall Rocks compared with Pittington Hill (8.46% and 13.17% respectively).

#### B. *P. lanceolata* L.

Table 2 shows a significant ( $P < 0.001$ ) variation in leaf area of populations of *P. lanceolata* L. collected from eight sites covering different altitudes and habitats. Within a habitat leaf area differed significantly: plants from the exposed Pittington Hill site had a mean leaf area of  $4.25 \pm 0.27 \text{ cm}^2$ , whereas those plants shaded by trees were larger leaved ( $10.6 \pm 1.01 \text{ cm}^2$ ). At Garrigil, a population growing on deeper soil was larger leaved ( $6.13 \pm 0.10 \text{ cm}^2$ ) whereas on the stony substrate, the leaves were on average smaller ( $1.81 \pm 0.10 \text{ cm}^2$ ). Leaves from plants at Widdybank Fell (529m) were very small, with a mean area of  $0.80 \pm 0.19 \text{ cm}^2$ .

**TABLE 2** Mean Leaf Area of populations of *P. lanceolata* L. from eight study sites of different altitude.

SITE	Altitude (m)	Area (cm <sup>2</sup> ) $\pm$ SE
Blackhall Rocks	10	$2.10 \pm 0.26$
Pittington E	120-150	$4.25 \pm 0.27$
Pittington S	120-150	$10.63 \pm 1.01$
Low Force	275	$3.63 \pm 0.33$
Widdybank Fell	529	$0.80 \pm 0.19$
Leadgate	350	$3.25 \pm 0.28$
Garrigil L	340	$6.13 \pm 0.10$
Garrigil S	340	$1.81 \pm 0.10$
		***

MANOVA showed highly significant differences in the Stomatal indices, stomatal frequency and guard cell length between and within sites. ( $P < 0.001$ ), see App. 2. Fig. 9 shows leaf SI's increase on both the adaxial and abaxial surface of *P. lanceolata* L. with increasing altitude: Blackhall ( $26.0\% \pm 0.5\%$ ); Widdybank Fell ( $33.5 \pm 0.6\%$ ), the exception is Pittington

**FIG. 9**            Mean Stomatal Index in leaves of  
*P. lanceolata* L. collected from five  
populations over a wide altitudinal and  
habitat range. ( $P < 0.001$ ) ANOVA.

**FIG. 10**          Mean Stomatal Numbers/mm<sup>2</sup> in leaves of  
*P. lanceolata* L. collected from five  
populations over a wide altitudinal and  
habitat range. ( $P < 0.001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) =  
counts from 25 fields of view.

**SITE:**            Blackhall Rocks (10m)  
                      Pittington Hill (120-150m)  
                      Low Force (275m)  
                      Leadgate (350m)  
                      Widdybank Fell (529m)



FIG. 9 **STOMATAL INDICES**  
**PLANTAGO LANCEOLATA L.**

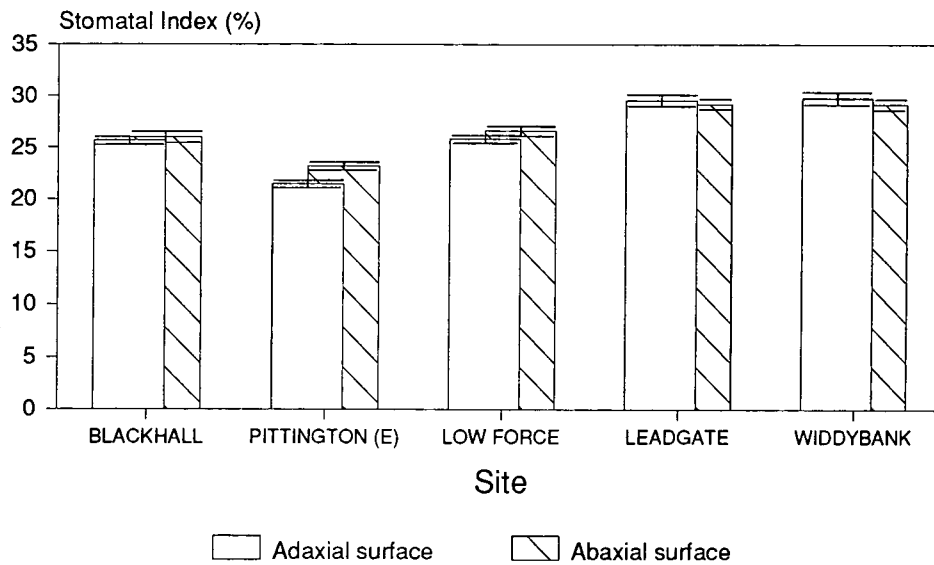
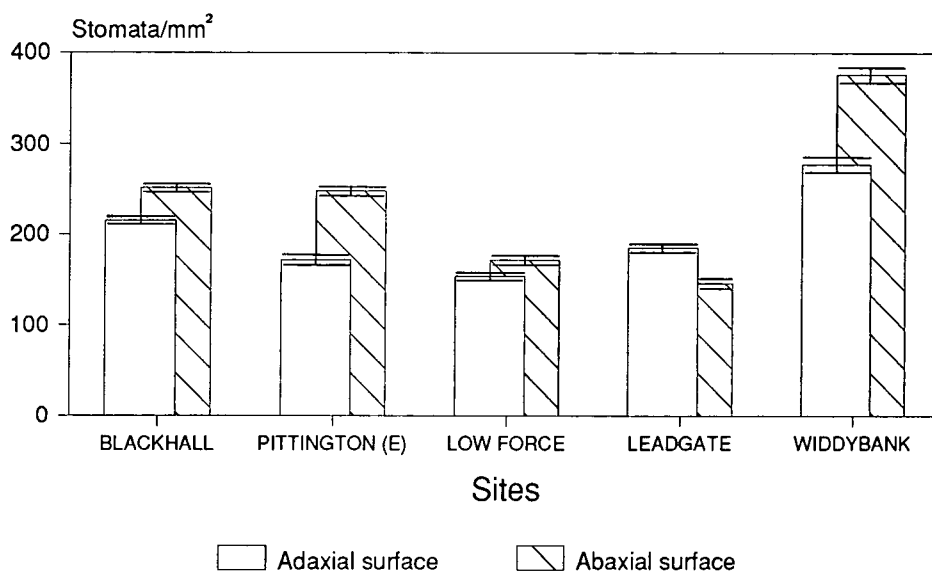


FIG. 10 **STOMATAL NUMBERS**  
**PLANTAGO LANCEOLATA L.**



**FIG. 11** Mean Stomatal Index in leaves of *P. lanceolata* L. collected from within habitats at two sites. Both the adaxial and abaxial leaf surface are shown. (P < 0.001) ANOVA.

**FIG. 12** Mean Stomatal Numbers/mm<sup>2</sup> in leaves of *P. lanceolata* L. collected from within habitats at two sites. Both the adaxial and abaxial surface are shown. (P < 0.001) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 fields of view.

Pittington	1 = Exposed habitat;	2 = Shaded habitat
Garrigil	1 = Stony soil;	2 = Deeper soil

FIG. 11 **PLANTAGO LANCEOLATA L.**  
WITHIN HABITAT VARIATION

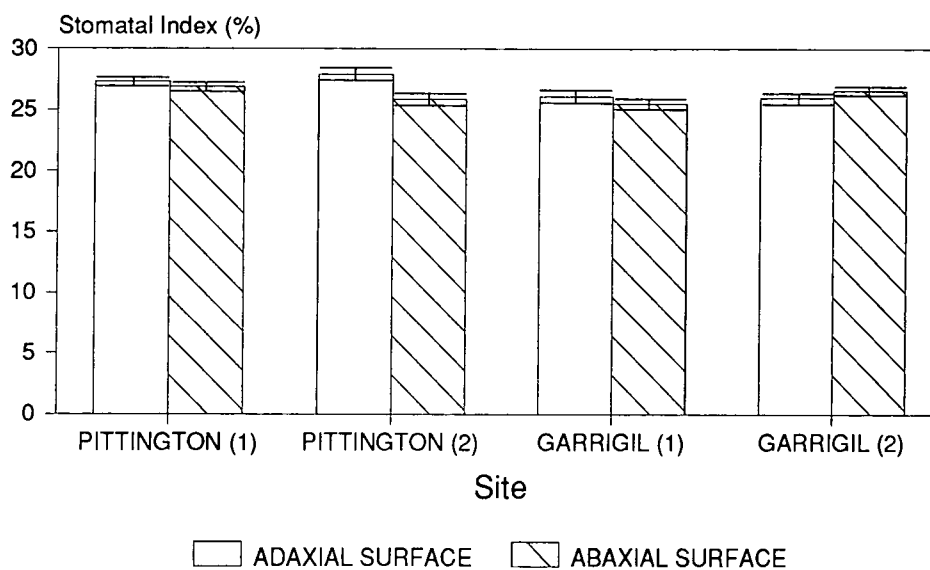
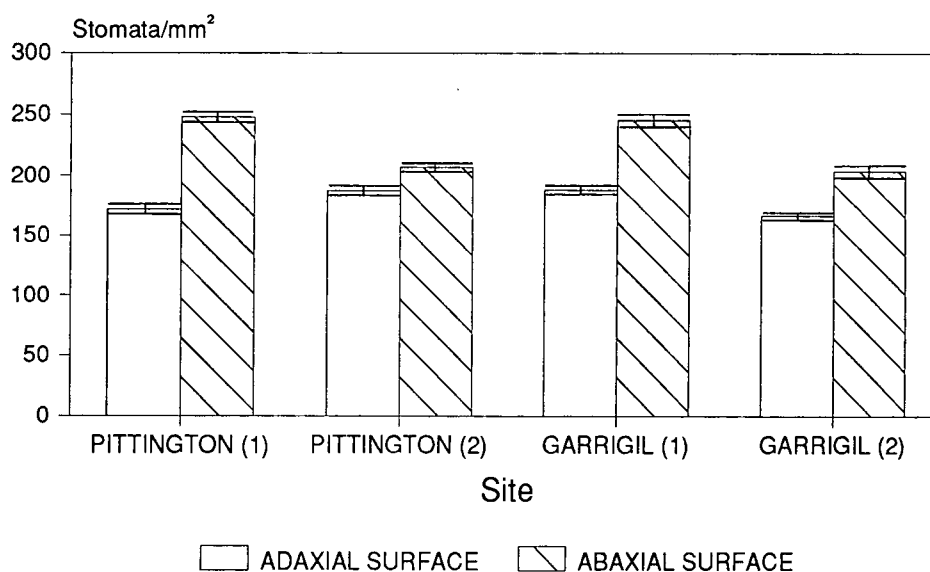


FIG. 12 **PLANTAGO LANCEOLATA L.**  
WITHIN HABITAT VARIATION



**FIG. 13** Mean Guard Cell Length in leaves of *Plantago lanceolata* L. collected from eight populations over a wide altitudinal range. (P< 0.001) ANOVA.

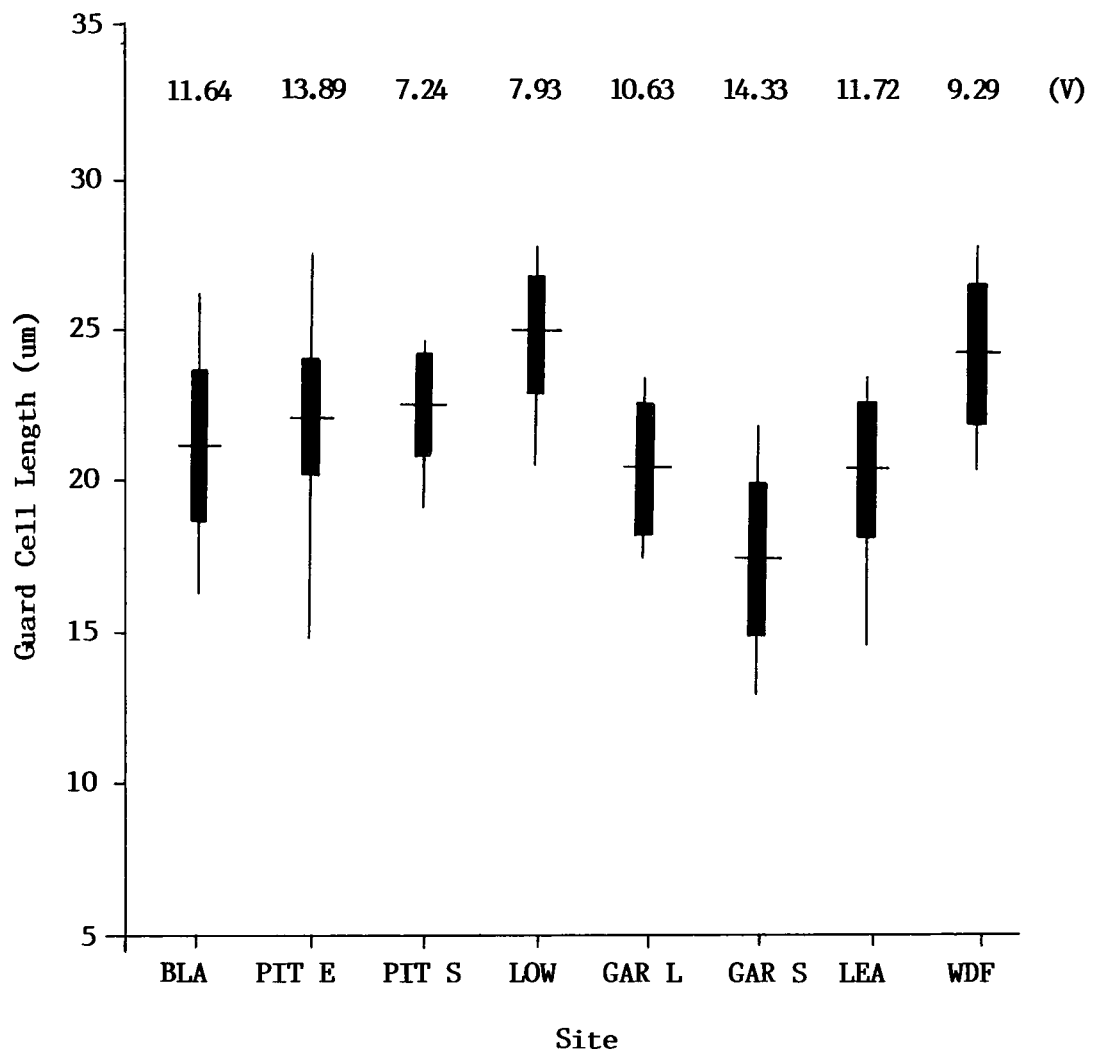
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N (Sample Number) = measurements from 25 guard cells.

SITE: Blackhall Rocks (10m)  
Pittington Hill E (120-150m)  
Pittington Hill S (120-150m)  
Low Force (275m)  
Garrigil L (340m)  
Garrigil S (340m)  
Leadgate (350m)  
Widdybank Fell (529m)

Garrigil L = Large leaved  
Garrigil S = Small leaved  
Pittington E = Exposed  
Pittington S = Shaded

FIG. 13

## GUARD CELL LENGTH

*Plantago lanceolata* L.

Hill (E), whose plants had a lower leaf SI. The SE of the means are quite small (between 0.4% - 0.6%). No significant differences were observed in leaf SI's between the upper and lower leaf surfaces except at the Widdybank Fell site and Pittington (S) site ( $P < 0.001$ ) as tested using T tests. (See App. 2).

Leaf Stomatal density (Fig. 10) decreased with altitude up to 350m (Leadgate). An increase in leaf stomatal density occurred in leaves of the Widdybank population, especially on the abaxial leaf surface. ( $376.5 \pm 7.9/\text{mm}^2$ ). Fig. 10 shows that more stomata occurred on the abaxial leaf surface in *P. lanceolata* L. except from the Leadgate population. Differences between the upper and lower surfaces were significant at the 0.1% level of probability (T-test, see App. 2). Within-habitat variation is shown in Figs. 11 and 12 for two sites. At Garrigil no significant variation occurred in the SI (Fig. 11). Stomatal density (Fig. 12) was significantly higher in the small leaved plants on stony soil ( $245.5 \pm 4.5/\text{mm}^2$  Bottom of leaf;  $188.6 \pm 4.4/\text{mm}^2$  Top of leaf) compared with the larger leaved plants on deeper soil ( $203.7 \pm 4.5/\text{mm}^2$  Bottom of leaf;  $166.9 \pm 2.9/\text{mm}^2$  Top of leaf).

At Pittington Hill, very little variation in the SI's were found on either surface, which was not significant (Fig 11). Leaves under exposure (Fig. 12) had significantly ( $P < 0.001$ ) greater stomatal density ( $248 \pm 4.8/\text{mm}^2$ ) compared with stomatal density in leaves from a shaded habitat ( $207 \pm 3.97/\text{mm}^2$ ) on the abaxial leaf surface. No significant difference was found on the adaxial surface. The SE of the mean stomatal density is fairly high on both leaf surfaces from Widdybank.

Variation in guard cell length both within and between sites is shown in Fig. 13. Leaves from plants at Garrigil and Leadgate had smaller guard cell lengths compared with those from Blackhall Rocks, Low Force and Widdybank. The range in size is large at the Pittington Exposed population. The Coefficient of Variation in size is smaller (7.24%, 7.93% and 10.63% respectively) in leaves from Blackhall, Low Force and Widdybank Fell, whose populations all had larger guard cells. Within habitat, at Garrigil, guard cells were significantly ( $P < 0.001$ ) shorter ( $17.52 \pm 0.50 \text{ um}$ ) in small leaves collected from plants on stony shallow soil compared with larger leaves collected from plants on deeper soil ( $20.70 \pm 0.44 \text{ um}$ ). Within habitat at Pittington no significant variation in guard cell length was observed. (See App. 2).

C. *A. pseudoplatanus* L.

Leaves selected from trees of *A. pseudoplatanus* L. at three sites of increasing altitude showed significant differences in size ( $P < 0.001$ ). Leaves from the lowland site, Frankland and Kepier, ( $49.6 \pm 2.74 \text{ cm}^2$ ) and Low Force ( $51.60 \pm 2.53 \text{ cm}^2$ ) were on average significantly smaller compared to the Hartside leaves ( $58.13 \pm 2.49 \text{ cm}^2$ ) - Scheffe ( $P < 0.05$ ).

**TABLE 3** Mean Leaf Area of the leaves taken from *A. pseudoplatanus* L. trees at three study sites of different altitude.

SITE	Altitude (m)	Area ( $\text{cm}^2$ ) $\pm$ SE
Frankland & Kepier	35-65	$49.61 \pm 2.74$
Low Force	275	$51.60 \pm 2.53$
Hartside	350	$58.13 \pm 2.49$ ***

Results of MANOVA showed highly significant differences in the leaf SI, stomatal density and guard cell length between sites ( $P < 0.001$ ). Leaf SI (Fig. 14) increases with increasing altitude (Frankland  $8.2\% \pm 0.3\%$ ; Hartside  $12.7\% \pm 0.3\%$ ). The Hartside population is significantly different ( $P < 0.05$ ) from Frankland and Low Force (Scheffe). The SE of the mean is small (0.3%).

The number of stomata/ $\text{mm}^2$  increases with increasing altitude (Fig. 15): Results from Frankland and Kepier ( $110.5 \pm 4.8/\text{mm}^2$ ) were significantly different at the 5% level from Low Force and Hartside ( $154.7 \pm 7.6/\text{mm}^2$  and  $161.2 \pm 5.0/\text{mm}^2$ ) respectively (Scheffe). Mean leaf guard cell length, range and variability about the mean are shown in Fig. 16. An initial decrease in the length is shown with increasing altitude. (Frankland -  $22.27 \text{ um}$  and Low Force -  $16.8 \text{ um}$  respectively). Results from Frankland were significantly different from Low Force and Hartside; no difference in guard cell length was found between the Low Force and Hartside leaves ( $16.8 \text{ um}$  and  $16.6 \text{ um}$ ) respectively at the 5% level of probability. The Range in leaf guard cell length from Frankland and Kepier is large but the variation in size was less (20.34%).

**FIG. 14**      Mean Stomatal Index in leaves of  
*A. pseudoplatanus* L. collected from three  
populations growing at different altitudes.  
( $P < 0.001$ ) ANOVA.

**FIG. 15**      Mean Stomatal Numbers/mm<sup>2</sup> in leaves of  
*A. pseudoplatanus* L. collected from three  
populations growing at different altitudes.  
( $P < 0.001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) =  
counts from 25 half fields of view.

**SITE:**            Frankland & Kepier (35-65m)  
                      Low Force (275m)  
                      Hartside (350m)



FIG. 14 **STOMATAL INDICES**  
**ACER PSEUDOPLATANUS L.**

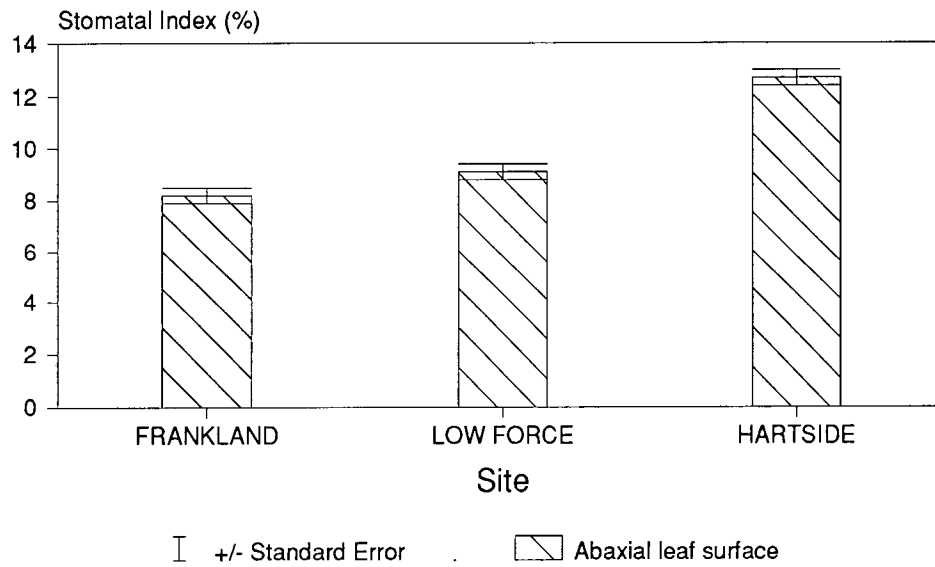
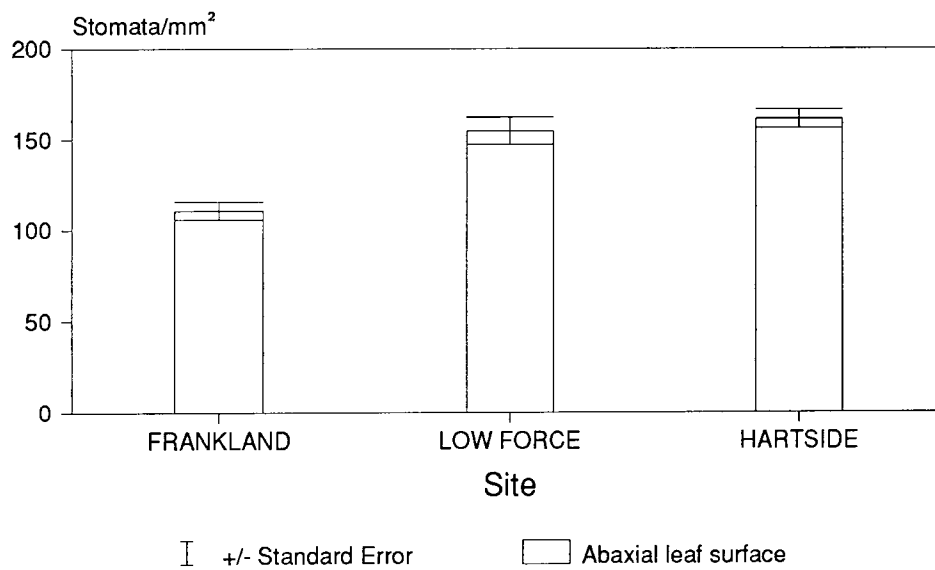


FIG. 15 **STOMATAL NUMBERS**  
**ACER PSEUDOPLATANUS L.**

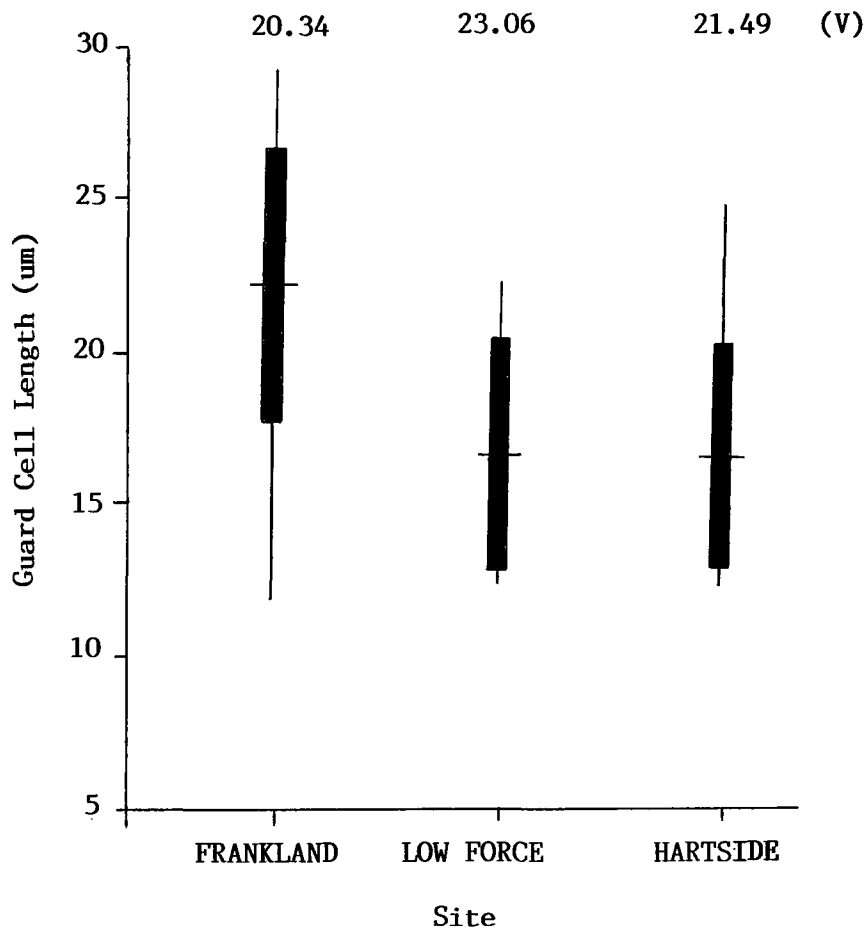


**FIG. 16**      Mean Guard Cell Length in leaves of  
*A. pseudoplatanus* L. collected from three  
populations growing at different altitudes.  
( $P < 0.001$ ) ANOVA.

The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

**SITE:**            Frankland and Kepier (35-65m)  
                      Low Force (275m)  
                      Hartside (350m)

FIG. 16 GUARD CELL LENGTH

*Acer pseudoplatanus* L.

### 3.1.2 Transplants

Leaf area varied significantly ( $P < 0.000$ ) between the transplanted seedlings at three sites (Table 4). Seedlings at Gilesgate Moor had significantly larger leaves (Scheffe).

**TABLE 4** Mean Leaf Area *A. pseudoplatanus* L. seedlings transplanted at three study sites of different altitude.

SITE	Altitude (m)	Area (cm <sup>2</sup> ) $\pm$ SE
Gilesgate Moor	85	57.28 $\pm$ 2.16
Esh	210	50.49 $\pm$ 1.60
Hartside	350	52.80 $\pm$ 1.68
n = 5		***

Transplanted seedlings of *A. pseudoplatanus* L. show a significant ( $P < 0.001$ ) increase in both leaf SI and the number of stomata/mm<sup>2</sup> with increasing altitude (Figs. 17 and 18 respectively). Mean leaf SI (Fig. 17) of the Gilesgate population (13.3%  $\pm$  0.3%) differs significantly from Esh (14.2%  $\pm$  0.3%) and Hartside (16.5%  $\pm$  0.4%). Leaf stomatal density (Fig. 18 in the Gilesgate population (207.04/mm<sup>2</sup>) differ significantly from Esh (233.2/mm<sup>2</sup>) and Hartside (280.7/mm<sup>2</sup>) leaves (Scheffe). Guard cell length decreased from 18.87  $\pm$  0.39  $\mu$ m to 14.88  $\pm$  0.50  $\mu$ m with increasing altitude in the transplanted seedlings (Fig. 19). The Coefficient of Variation or spread also increases with increasing altitude (10.23%, 14.70% and 16.94% respectively). The range in guard cell length was smaller in the leaves from Gilesgate.

**FIG. 17**      Mean Stomatal Index in leaves of  
*A. pseudoplatanus* L. seedlings transplanted at  
sites of three different altitudes.  
( $P < 0.0001$ ) ANOVA.

**FIG. 18**      Mean Stomatal Numbers/mm<sup>2</sup> in leaves of  
*A. pseudoplatanus* L. seedlings transplanted at  
sites of three different altitudes.  
( $P < 0.0001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (sample number) = counts from 25 half fields of view.

SITES:          Gilesgate (83m)  
                  Esh (210m)  
                  Hartside (350m)

FIG. 17

# TRANSPLANTS ACER PSEUDOPLATANUS L. STOMATAL INDICES

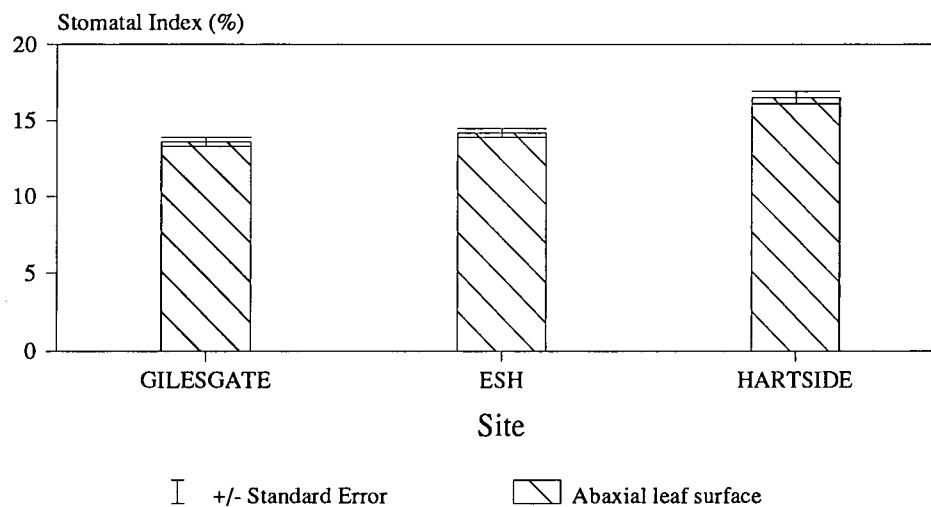
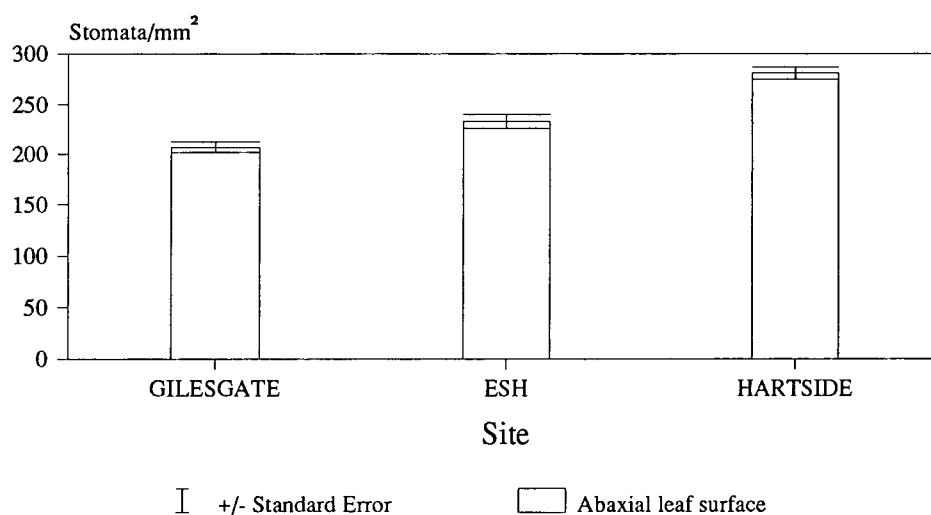


FIG. 18

# TRANSPLANTS ACER PSEUDOPLATANUS L. STOMATAL NUMBERS



**FIG. 19**      Mean Guard Cell Length in leaves of  
*A. pseudoplatanus* L. seedlings transplanted at  
sites of three different altitudes.  
( $P < 0.0001$ ) ANOVA.

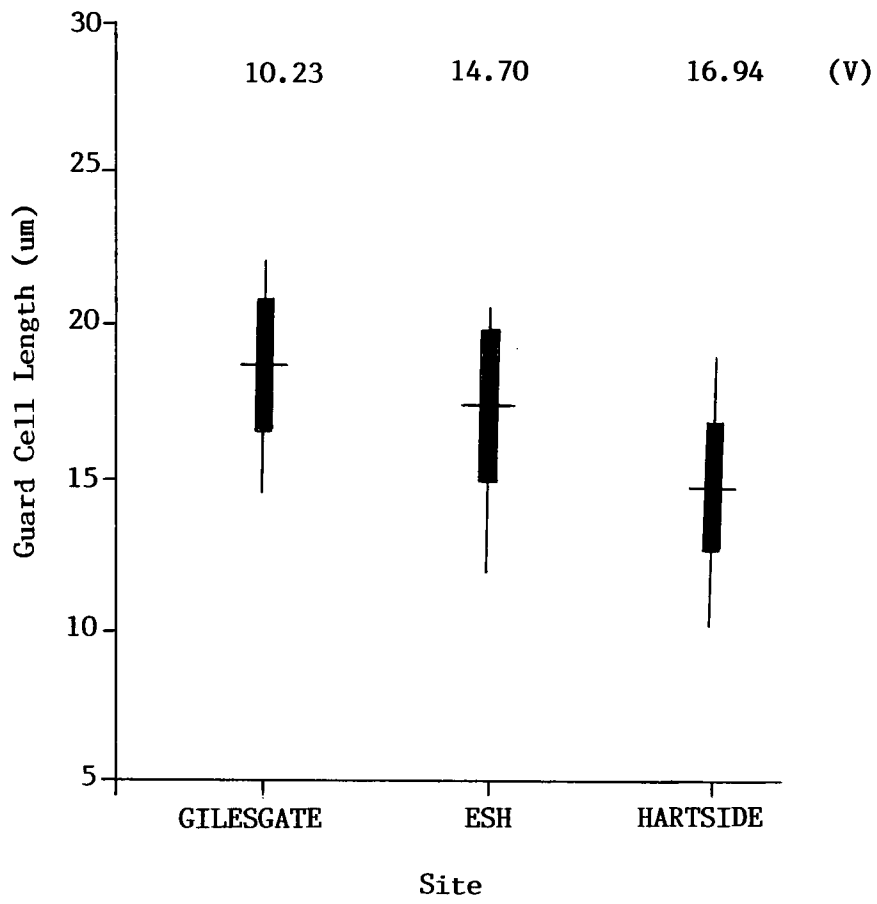
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

SITES:          Gilesgate (83m)  
                    Esh (210m)  
                    Hartside (350m)

FIG. 19

## TRANSPLANTS

## GUARD CELL LENGTH

*Acer pseudoplatanus* L.



### 3.1.3 Moisture Content and Depth of Soil

**TABLE 5** SOIL DEPTH AND PERCENTAGE MOISTURE CONTENT OF THE SOILS

SITE	ALTITUDE (METRES)	SPECIES	SOIL DEPTH (INCHES)	MOISTURE CONTENT OF OVEN DRIED SOIL EXPRESSED ON WET BASIS (%)
BLACKHALL	10	1	5"	58.20%
PITTINGTON E	150	1	1"	15.43%
LOW FORCE	275	1	<1"	-
WIDDYBANK	529	1	1"	35.44%
BLACKHALL	10	2	5"	58.20%
PITTINGTON E	150	2	1"	15.43%
PITTINGTON S	150	2	3"	24.20%
LOW FORCE	275	2	6"	32.00%
WIDDYBANK	529	2	1"	35.44%
LEADGATE	350	2	3"	45.02%
GARRIGIL L	340	2	3"	31.03%
GARRIGIL S	340	2	1"	14.47%
FRANKLAND	60	3	4"	41.03%
LOW FORCE	275	3	7"	38.33%
HARTSIDE	350	3	7"	42.50%

PITTINGTON E or S = Exposed or Shaded  
 GARRIGIL L or S = Large or Small leaves  
 SPECIES  
     1 = *Acer pseudoplatanus* L.  
     2 = *Plantago lanceolata* L.  
     3 = *Sesleria caerulea* L.

Table 5 shows the soil depth recorded at each site where plants were collected, together with the moisture content, expressed on a wet basis (%). Both Soil depth and moisture content varied considerably with altitude. The deeper soils appear to hold more moisture, especially if peaty; where soil depth is shallow, the soil is fairly dry. A Multivariate approach: Stepwise Multiple regressions of the independent variables: moisture content, altitude and soil depth with the dependent variables stomatal densities, stomatal indices and guard cell length were run for each species to determine the relative importance of environmental variables on these parameters. (App. 2). This statistical test shows which independent variable was the most probable predictor of each dependent variable. Independent variables were entered stepwise, second and subsequent entries affecting the residual variation until no further variation could be explained. The existence of a linear relationship between X and Y was determined by a T-test.

*A. pseudoplatanus L.*

Soil depth was the most important predictor of stomatal density at the Sycamore sites, being significantly correlated ( $R = 0.61$ ,  $P < 0.0001$ ), with 36.8% of the variation occurring being explained by soil depth alone. A significant positive linear relationship was found between stomatal density and soil depth (Regression equation:  $\text{Number} = 47.2 + 15.8 (\text{Depth})$ ). Altitude alone was an important predictor of the SI ( $R = 0.67$ ,  $P < 0.0001$ ) the residual variation being explained by soil depth ( $R = 0.81$ ,  $P < 0.001$ ). The SI was significantly positively linearly related with altitude and negatively related with soil depth (Regression Equation:  $\text{Index} = 0.17 + 4.75 (\text{Alt}) - 0.030 (\text{Depth})$ ). Altitude was also a good predictor of guard cell length ( $R = 0.50$ ,  $P < 0.0001$ ), explaining 24% of the variation in guard cell size. A significant linear negative relationship of guard cell length with altitude is shown (Regression Equation:  $\text{Guard} = 23.27 - 0.0197 \text{ Alt}$ ). Depth and altitude were found to covary. (App. 2).

*S. caerulea L.*

Soil depth was selected as the best predictor of stomatal density. Soil moisture was the second important variable explaining the residual variation and altitude the third explaining the variation left after the first two steps. Correlation coefficients were significant ( $R = 0.75$ ,  $R = 0.84$ ,  $R = 0.94$  increasing with the addition of each variable). Fifty-six per cent of the variation can be explained by soil depth alone, 71% by both soil depth and moisture and 90% by all three. Stomatal density was significantly negatively related both with soil depth and altitude but positively related with moisture (Regression Equation:  $\text{Number} = 31.2 - 18.0 \text{ Depth} + 16.2 \text{ Moist} - 12.8 \text{ Alt}$ ). However the Correlation Coefficient (0.9) showed multicollinearity of two independent variables, moisture and depth, which may account for the inflated variances.

Soil depth was found to be the most important predictor of guard cell length ( $P < 0.001$ ) with 33.8% of the variation being explained by this environmental variable ( $R = 0.85$ ). A significant linear relationship existed between guard cell length and soil depth (Regression Equation:  $\text{Guard} = 15.390 + 1.7809 (\text{Depth})$ ). Moisture and soil depth appeared to be intercorrelated with high Correlation Coefficients.

*P. lanceolata L.*

Soil depth was selected as the more likely predictor of stomatal density ( $R = 0.50$ ,  $P < 0.0001$ ). Soil moisture was entered as the second environmental variable affecting the residual variation ( $R = 0.58$ ,  $P < 0.0001$ ). Together, soil depth and soil moisture account for 33.78% of

the variation in the data. Stomatal density and soil depth were negatively linearly related, stomatal density and soil moisture were positively linearly related. Regression Equation: Number =  $252.7 - 28.30 (\text{Depth}) + 1.87 (\text{Moist})$ . Altitude appeared to be a significant predictor of the SI ( $R = 0.51$ ,  $P < 0.0001$ ). The residual variation was explained by soil moisture ( $R = 0.56$ ,  $P < 0.0001$ ) and soil depth ( $R = 0.61$ ,  $P < 0.0001$ ), all three accounting for 38% of the variation. (26% altitude; 31% altitude and soil moisture; 38% altitude, soil moisture and soil depth). The regression Equation: Index =  $0.23 + 1.02 (\text{Alt}) + 0.001 (\text{Moist}) - 0.006 (\text{Depth})$ ; shows the SI to be significantly positively related with altitude and soil moisture but negatively related with soil depth.

Soil depth was selected as a significant ( $P = 0.0001$ ) predictor of guard cell length; altitude was entered as a second step explaining the residual variation. Together  $R = 0.32$ ; 7% of the variation being explained by soil depth alone and 10% by both soil depth and altitude. A significantly ( $P < 0.001$ ) positive linear relationship between guard cell length and soil depth and guard cell length and altitude was found. (Regression equation: Guard =  $18.76 + 0.64 (\text{Depth}) + 0.004 (\text{Alt})$ ).

### 3.2 Experimental Studies

#### 3.2.1 Sycamore Seedling Variation

Leaf area (Table 6) increased significantly with increasing height in the seedling ( $P < 0.000$ ).

**TABLE 6** Leaf Area of successive leaves of *A. pseudoplatanus* L. seedlings

POSITION ON SEEDLING	Area (cm <sup>2</sup> ) of each leaf		Plant 3	Plant 4	Mean Area (cm <sup>2</sup> ) $\pm$ SE
	Plant 1	Plant 2			
Bottom	22.81	25.26	28.48	18.55	$23.78 \pm 2.09$
Middle	28.68	30.29	36.06	28.64	$30.91 \pm 1.76$
Top	41.23	46.77	44.26	41.65	$43.48 \pm 1.29$
n = 5					***

Fig. 20 shows leaf SI's increase successively in the leaves of four *A. pseudoplatanus* L. seedlings up the stem. In plant 1 the mean SI was  $10.2\% \pm 0.4\%$  in the basal leaf. The proportion rose to  $13.4\% \pm 0.3\%$  in the Middle leaf, and to  $15.5\% \pm 0.4\%$  in the top leaf. The number of stomata per mm<sup>2</sup> of leaf surface in plant 1 (Fig. 21) increases up the stem in

**FIG. 20**      Mean Stomatal Index determined in successive leaves of *A. pseudoplatanus* L. seedlings up the stem.

Up seedlings:                      ( $P < 0.001$ )  
Between seedlings:              ( $P = 0.012$ )

**FIG. 21**      Mean stomatal Numbers/mm<sup>2</sup> determined in successive leaves of *A. pseudoplatanus* L. seedlings up the stem.

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 half fields of view on one leaf from the Bottom, Middle and Top of each seedling.

FIG. 20 **SYCAMORE SEEDLINGS**  
**STOMATAL VARIATION**

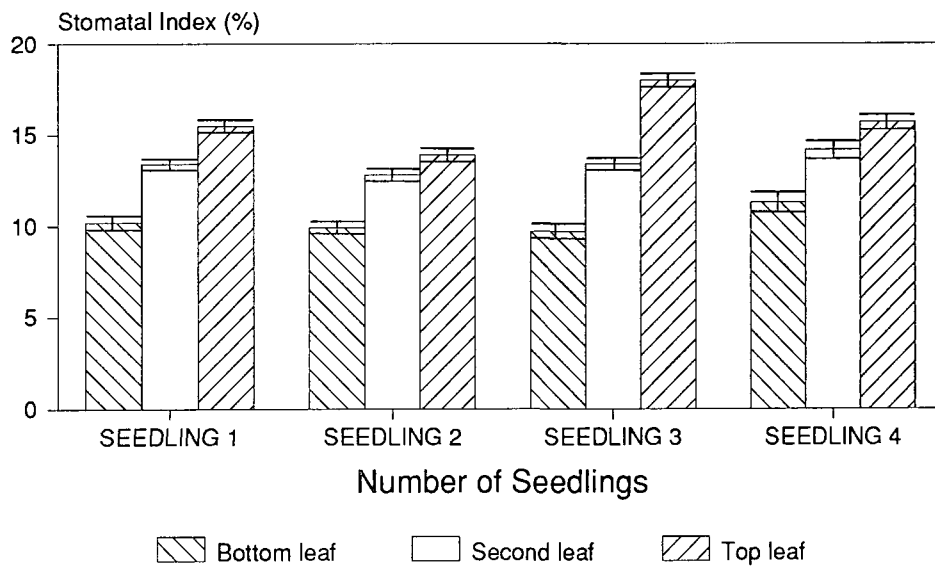
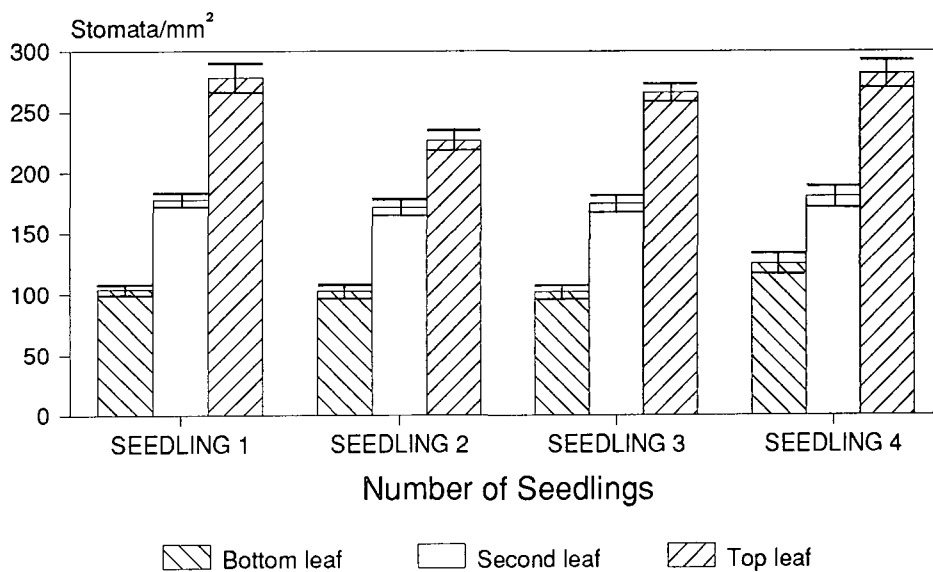


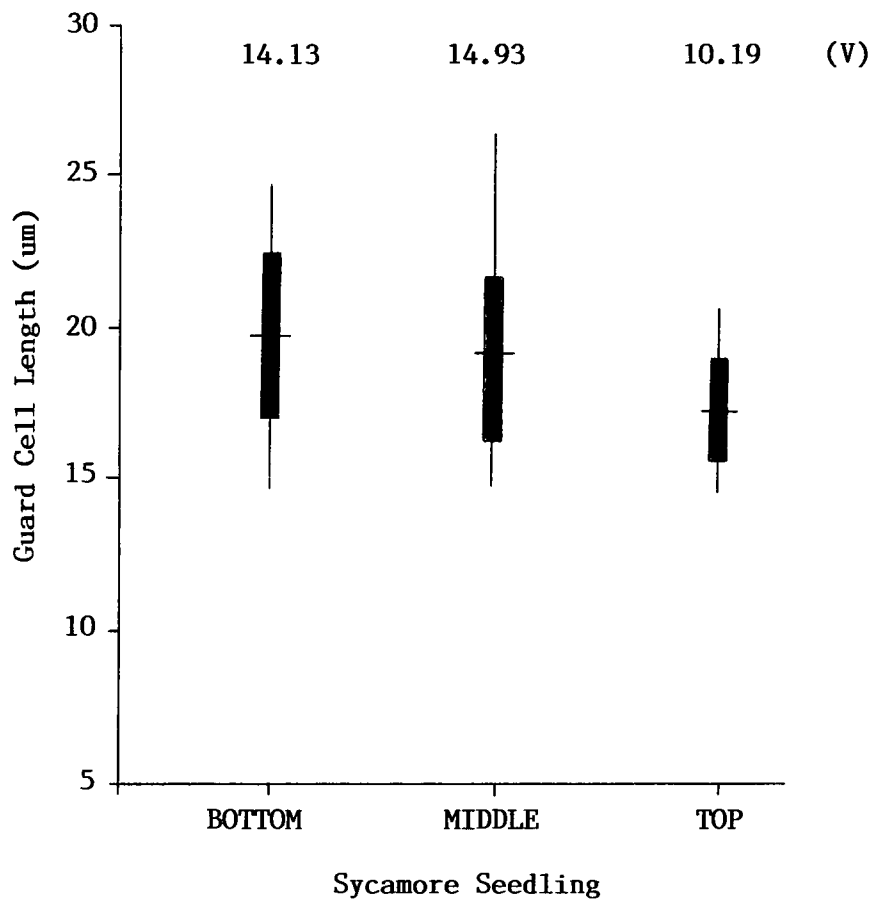
FIG. 21 **SYCAMORE SEEDLINGS**  
**STOMATAL VARIATION**



**FIG. 22**      Mean Guard Cell Length in successive  
leaves of one *A. pseudoplatanus* L. seedling.  
( $P < 0.001$ ) ANOVA.

The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

FIG. 22      S Y C A M O R E   S E E D L I N G  
G U A R D   C E L L   L E N G T H



successive leaves ( $103.9 \pm 4.4/\text{mm}^2$  Bottom leaf;  $177.6 \pm 5.4/\text{mm}^2$  Middle leaf;  $278.2 \pm 10.2/\text{mm}^2$  Top leaf). Similar patterns occurred in three other seedlings. Results were significant within and between plants at the 0.1% level of probability using ANOVA. Guard cell length, measured only in Plant 1 (Fig. 22), showed a decrease in length with increasing height ( $19.93 \pm 0.56 \mu\text{m}$ , basal leaf;  $17.11 \pm 0.34 \mu\text{m}$ , top leaf).

### 3.2.2 Shading Stress

Leaf area increased very significantly ( $P < 0.0001$ ) in *A. pseudoplatanus* L. with increasing shade (Table 7). Differences were significant ( $P < 0.05$ ) between each treatment (Scheffe).

**TABLE 7** Mean Leaf Area of youngest leaves of *A. pseudoplatanus* L. under varying shading levels.

TREATMENT	Area ( $\text{cm}^2$ ) $\pm$ SE
CONTROL	$37.45 \pm 2.41$
40% SHADE	$44.73 \pm 1.65$
60% SHADE	$50.71 \pm 2.06$
	****
n = 5	

Shading significantly ( $P < 0.0001$ ) reduced both the leaf SI from  $16.4\% \pm 0.4\%$  in the control leaves to  $13.0\% \pm 0.5\%$  in leaves under 60% shade, and the density of stomata from  $230 \pm 7.9/\text{mm}^2$  in control leaves to  $166.9 \pm 6.3/\text{mm}^2$  under 60% shade (Figs. 23 and 24 respectively and App. 1). Differences in leaf stomatal densities and indices lay in the control leaves which varied significantly from these parameters measured in the leaves under 40% and 60% shade ( $P < 0.05$ ). No significant difference was found in either parameters between the 40% and 60% shaded leaves. (Sheffe).

Fig. 25 shows an increase in mean guard cell length in leaves under 60% shade ( $+0.9\mu\text{m}$ ). This effect was not statistically significant ( $P = 0.4362$ ) The Coefficient of Variation or spread is higher in the control leaves as is the Standard Deviation (SD).



**FIG. 23**      Mean Stomatal Index in leaves of  
*A. pseudoplatanus* L. grown under different  
shade levels. ( $P < 0.001$ ) ANOVA.

**FIG. 24**      Mean Stomatal Numbers/mm<sup>2</sup> in leaves of  
*A. pseudoplatanus* L. grown under different  
shade levels. ( $P < 0.001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 fields of view.

TREATMENT: Control	= Almost 100% of light in growth room
40% shade	= 60% light
60% shade	= 40% light

FIG. 23 **ACER PSEUDOPLATANUS L.**  
EFFECT OF SHADING  
ON THE STOMATAL INDEX

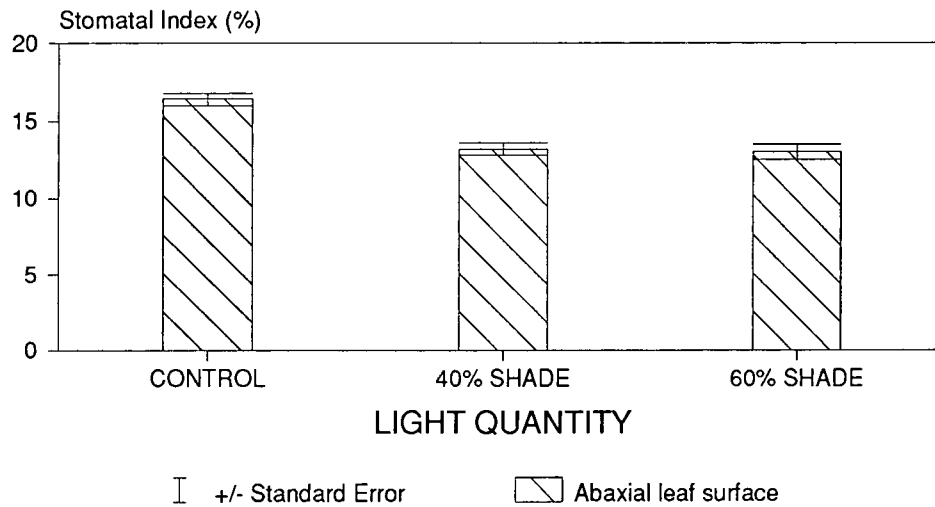
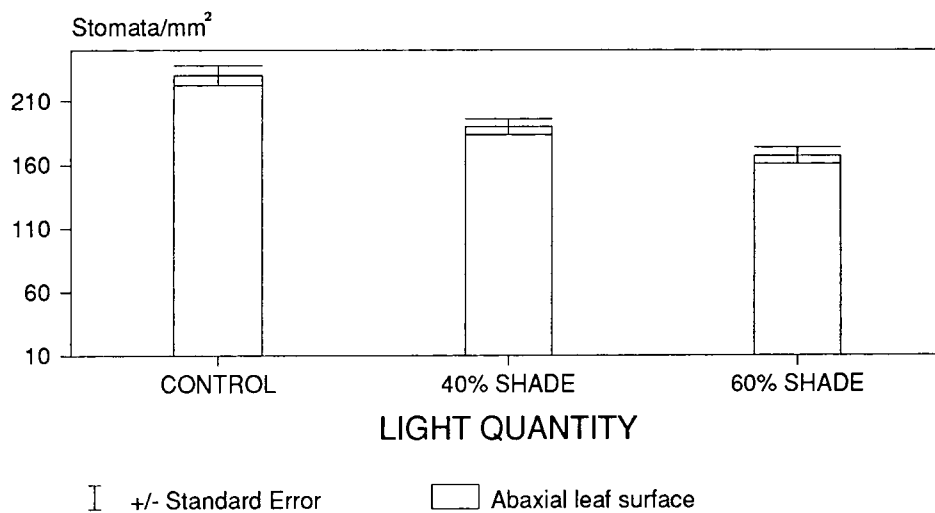


FIG. 24 **ACER PSEUDOPLATANUS L.**  
EFFECT OF SHADING  
ON STOMATAL NUMBERS



**FIG. 25**      Mean Guard Cell Length in leaves of  
*A. pseudoplatanus* L. grown under different  
shade levels. (P = 0.4362) ANOVA.

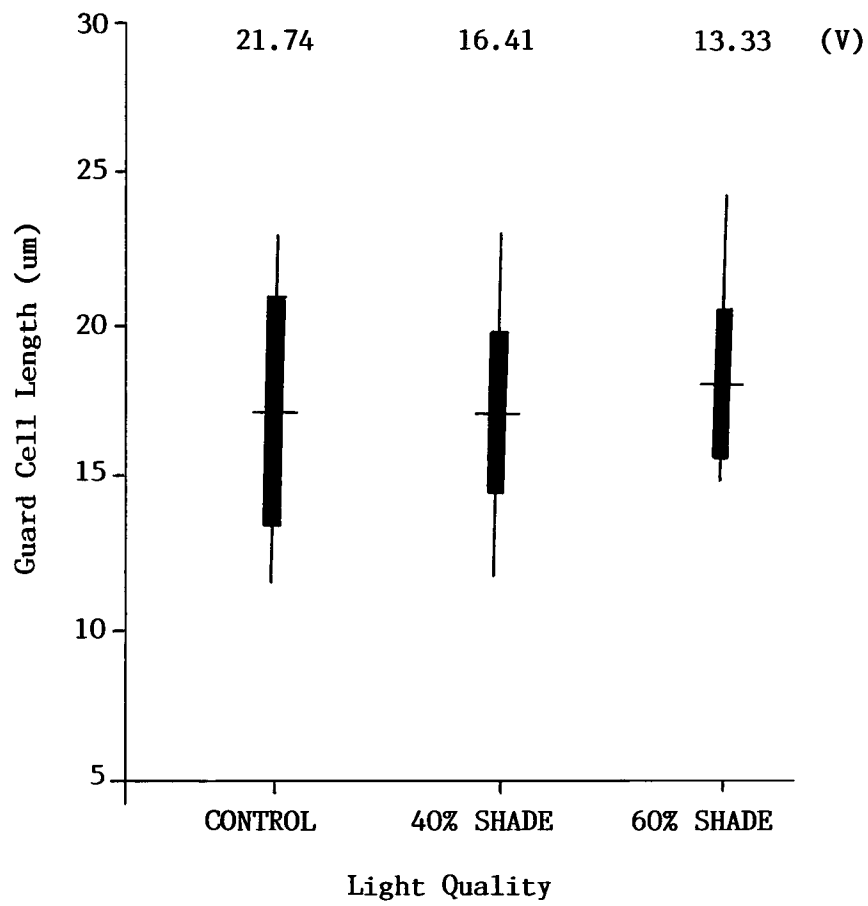
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

TREATMENT: Control	= Almost 100% light in growth room
40% Shade	= 60% light
60% Shade	= 40% light

FIG. 25

## G U A R D   C E L L   L E N G T H

Effect of shading on  
*Acer pseudoplatanus* L.



### 3.2.3 Water Stress

A1. *Sesleria caerulea* L.  
(Blackhall Rocks)

Table 8 shows leaf area of the leaf samples. A significant difference in size was recorded ( $P = 0.0009$ ); leaf area decreasing with increasing water stress.

**TABLE 8** Mean Leaf Area of youngest leaves of *S. caerulea* L. plants under differing watering regimes.

TREATMENT	Area (cm <sup>2</sup> ) $\pm$ SE
CONTROL	1.61 $\pm$ 0.17
EVERY 4 DAYS	1.47 $\pm$ 0.11
EVERY 7 DAYS	1.31 $\pm$ 0.12
	***
n = 5	

Fig. 26 shows a significant decrease in the leaf SI with increasing water stress ( $P < 0.0001$ ). An SI of  $34.8\% \pm 0.9\%$  in controls fell to  $31.1\% \pm 0.6\%$  under 4-day water stress and to  $27.4\% \pm 0.6\%$  under 7-day water stress. Differences were not significant between control and 4-day stressed leaves at the 5% level. A highly significant increase in stomatal density (Fig. 27) is shown with increasing water stress ( $P < 0.0001$ ). A mean stomatal density of  $115.4 \pm 4.4/\text{mm}^2$  of leaf surface in controls rose to  $195.6 \pm 4.5/\text{mm}^2$  under 4-day water stress and to  $215.6 \pm 4.2/\text{mm}^2$  under 7-day water stress. The differences were significant at the 5% level between all treatments (scheffe). (See Apps. 1 and 2).

A significant decrease in leaf guard cell length with increasing water stress is shown in Fig 28 ( $P < 0.0001$ ). The variation is small between the 4 and 7-day water stress treatments which was not statistically significant at the 5% level of probability (Scheffe). The minus SD exceeds the Range in the control.

**FIG. 26** Mean Stomatal Index in leaves of *S. caerulea* L. taken from plants subjected to different watering regimes. Plants were from a lowland, coastal population (Blackhall Rocks). ( $P < 0.0001$ ) ANOVA.

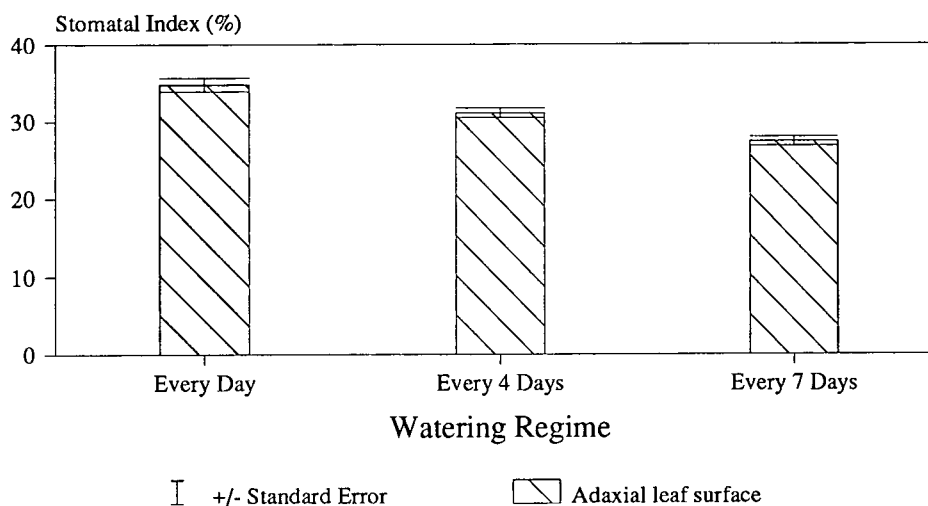
**FIG. 27** Mean Stomatal Numbers/mm<sup>2</sup> in leaves of *S. caerulea* L. taken from plants subjected to different watering regimes. Plants were from a lowland, coastal population (Blackhall Rocks). ( $P < 0.0001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 fields of view.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days

FIG. 26

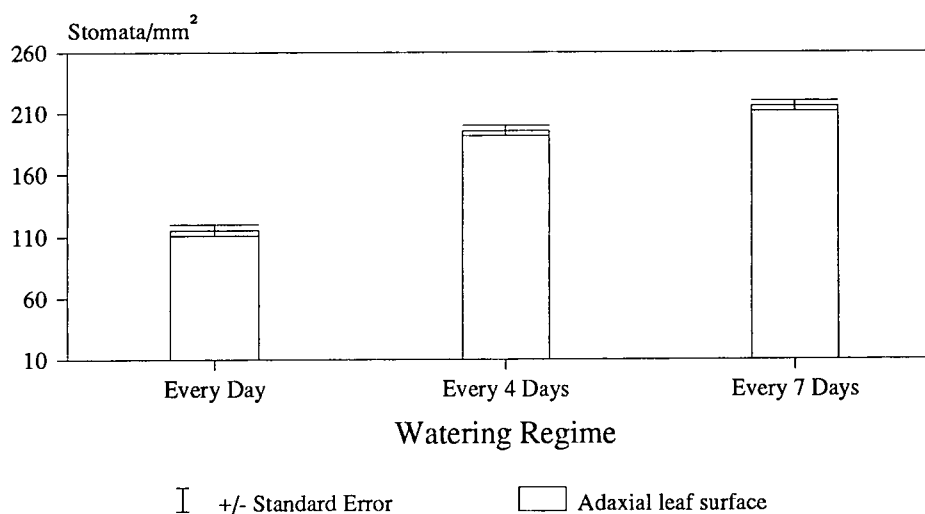
## SESLERIA CAERULEA L. EFFECTS OF WATERING REGIMES ON THE STOMATAL INDEX



Site: Blackhall Rocks

FIG. 27

## SESLERIA CAERULEA L. EFFECTS OF WATERING REGIMES ON STOMATAL NUMBERS



Site: Blackhall Rocks

**FIG. 28** Mean Guard Cell Length in leaves of *S. caerulea* L. taken from plants subjected to different watering regimes. Plants were from a lowland, coastal population (Blackhall Rocks). ( $P < 0.0001$ ) ANOVA.

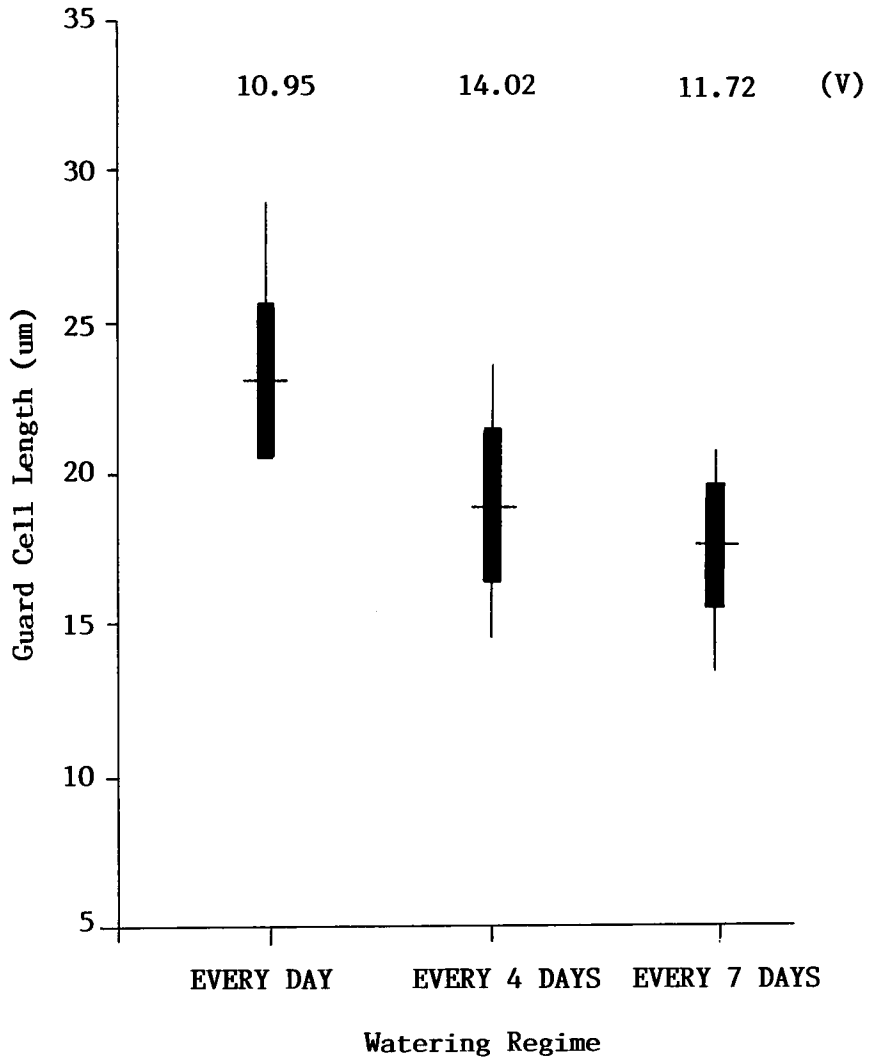
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N (Sample Number) = measurements from 25 guard cells.

TREATMENT: Control = Watered daily  
4 days = Watered every 4 days  
7 days = Watered every 7 days.



FIG. 28 G U A R D C E L L L E N G T H

Watering Regime Effects on

*Sesleria caerulea* L.

Site: Blackhall Rocks

A2. *Sesleria caerulea* L.  
(Widdybank Fell)

Table 9 shows no significant difference in leaf area ( $P=0.251$ ) between treatments in the leaves from the upland population (Widdybank Fell).

**TABLE 9** Mean Leaf Area of youngest leaves of *S. caerulea* L. plants under differing watering regimes.

TREATMENT	Area (cm <sup>2</sup> ) $\pm$ SE
CONTROL	$0.89 \pm 0.07$
EVERY 4 DAYS	$0.82 \pm 0.11$
EVERY 7 DAYS	$0.82 \pm 0.04$
	NS
n = 5	

A significant ( $P < 0.0001$ ) decrease in the leaf SI with increasing water stress can be seen in Fig. 29. The proportion of stomata recorded in control leaves ( $30.8\% \pm 0.6\%$ ) fell to  $28.4\% \pm 0.4\%$  under 4-day water stress treatment and to  $26.8\% \pm 0.6\%$  under 7-day stress treatment (Fig. 29).

Stomatal density (Fig. 30) increased significantly ( $P < 0.0001$ ) with increasing water stress from  $292.9 \pm 6.0/\text{mm}^2$  in control leaves to  $314.7 \pm 5.2/\text{mm}^2$  in 4-day water stressed leaves to  $349.0 \pm 11.4/\text{mm}^2$  under 7-day stressed leaves. Differences in both parameters were highly significant as tested using ANOVA ( $P < 0.001$ ). The SE of the mean was high in the 7-day stressed leaves.

Guard cell length (Fig. 31) decreased significantly ( $P < 0.0001$ ) with increasing water stress. Under 7-day water stress treatment the Range in measurements is greater than those in control or 4-day stressed leaves.

**FIG. 29** Mean Stomatal Index in leaves of *S. caerulea* L. taken from plants subjected to different watering regimes. Plants were from an upland population (Widdybank Fell). ( $P < 0.0001$ ) ANOVA.

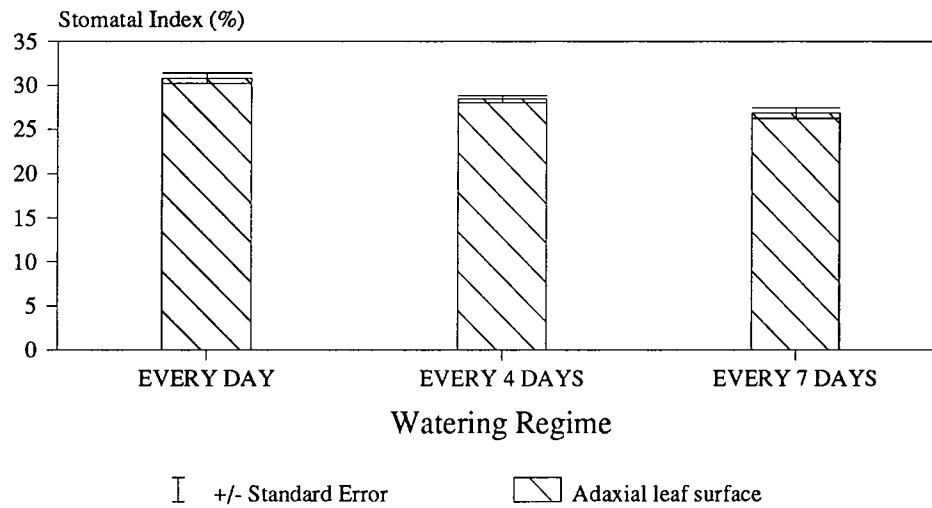
**FIG. 30** Mean Stomatal Numbers/mm<sup>2</sup> in leaves of *S. caerulea* L. taken from plants subjected to different watering regimes. Plants were from an upland population (Widdybank Fell). ( $P < 0.0001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 fields of view.

TREATMENT: Control	= Watered daily
4 Days	= Watered every 4 days
7 Days	= Watered every 7 days

FIG. 29

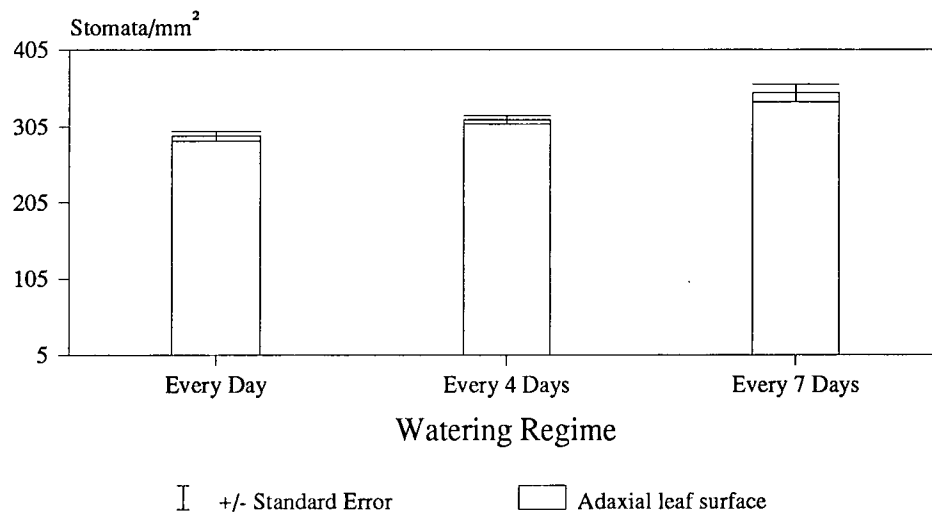
## SESLERIA CAERULEA L. EFFECTS OF WATERING REGIMES ON THE STOMATAL INDEX



Site: Widdybank Fell

FIG. 30

## SESLERIA CAERULEA L. EFFECTS OF WATERING REGIMES ON STOMATAL NUMBERS



Site: Widdybank Fell

**FIG. 31** Mean Guard Cell Length in leaves of *S. caerulea* L. taken from plants subjected to different watering regimes. Plants were from an upland population (Widdybank Fell). ( $P < 0.0001$ ) ANOVA.

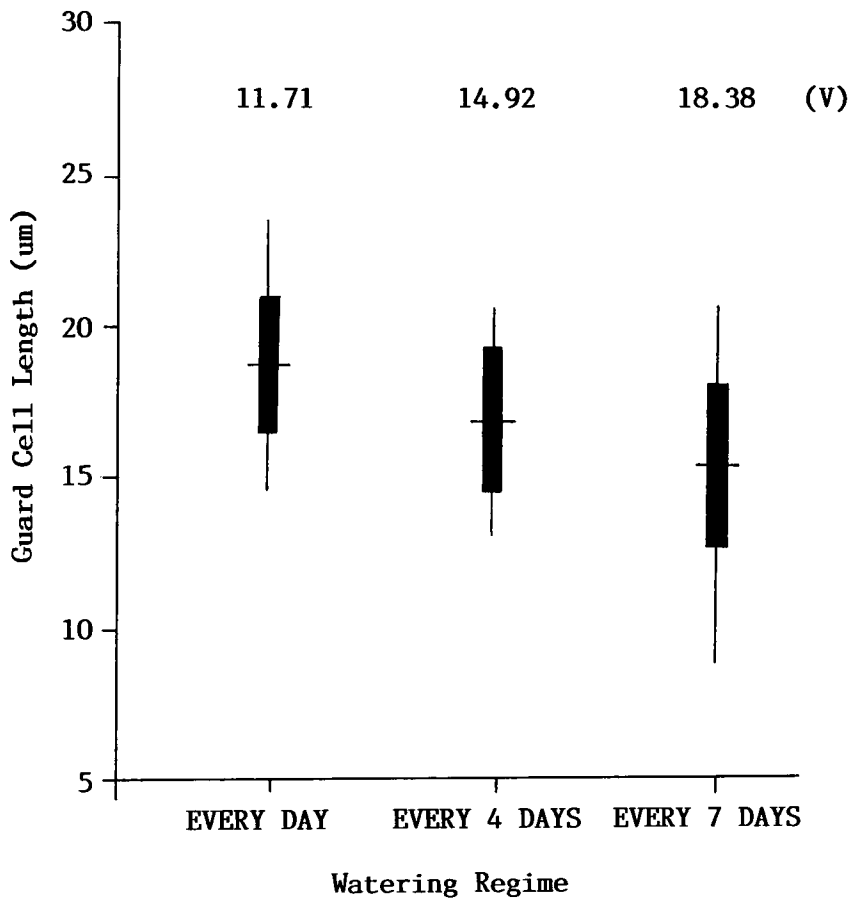
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N (Sample Number) = measurements from 25 guard cells.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days.

FIG. 31

## G U A R D   C E L L   L E N G T H

Watering Regime Effects on

*Sesleria caerulea* L.

Site: Widdybank Fell

B1. *P. lanceolata* L.  
(Blackhall Rocks)

A significant decrease in leaf area (Table 10) is shown with increasing water stress.  $P < 0.001$ ).

**TABLE 10** Mean Leaf Area of youngest leaves of *P. lanceolata* L. plants under differing watering regimes.

TREATMENT	Area (cm <sup>2</sup> ) $\pm$ SE
CONTROL	4.6 $\pm$ 0.33
EVERY 4 DAYS	4.0 $\pm$ 1.79
EVERY 7 DAYS	3.3 $\pm$ 1.49
	****
n = 5	

Mean leaf stomatal indices, stomatal density and guard cell lengths are shown in Figs. 32, 33 and 34 respectively. MANOVA and univariate F-Tests showed highly significant differences in all dependent variables between treatments ( $P \leq 0.0014$ ) on the abaxial leaf surface; on the adaxial leaf surface stomatal indices and density were also significantly different between treatments ( $P < 0.001$ ). The SI (Fig. 32 and App. 1) decreases with increasing water stress in both the abaxial and adaxial leaf surfaces in *P. lanceolata* L. from Blackhall Rocks. A mean leaf SI of  $26.8\% \pm 0.6\%$  observed in controls dropped to  $24.3\% \pm 0.2\%$  under 7-day water stress treatment on the abaxial leaf surface.

Leaf stomatal density (Fig. 33) increases with increasing water stress on both surfaces. Results of  $161.6 \pm 3.4/\text{mm}^2$  observed in controls rose to  $342.1 \pm 10.9/\text{mm}^2$  under 4-day water stress treatment, and to  $379.7 \pm 10.4/\text{mm}^2$  under 7-day water stress treatment. Mean guard cell length, measured only on the abaxial leaf surface, significantly decreased in size with increasing water stress (Fig. 34) from  $23.87 \pm 0.58 \text{ um}$  in control leaves to  $17.58 \pm 0.67 \text{ um}$  under 7-day water stress treatment (App 1). The SD and Coefficient of Variation in size was greater (18.94%) under 7-day stress treatment.

**FIG. 32** Mean Stomatal Index in the adaxial and abaxial surface of leaves of *P. lanceolata* L. subjected to different watering regimes. (Blackhall Rocks). ( $P = 0.0014$ ) ANOVA.

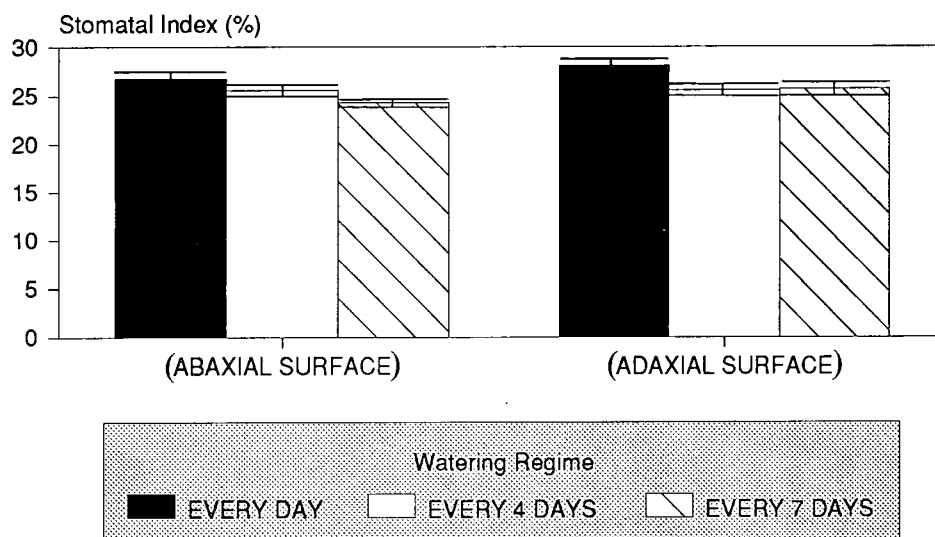
**FIG. 33** Mean Stomatal Numbers/mm<sup>2</sup> in the adaxial and abaxial surface of leaves of *P. lanceolata* L. subjected to different watering regimes (Blackhall Rocks). ( $P < 0.001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (sample number) = counts from 25 fields of view.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days

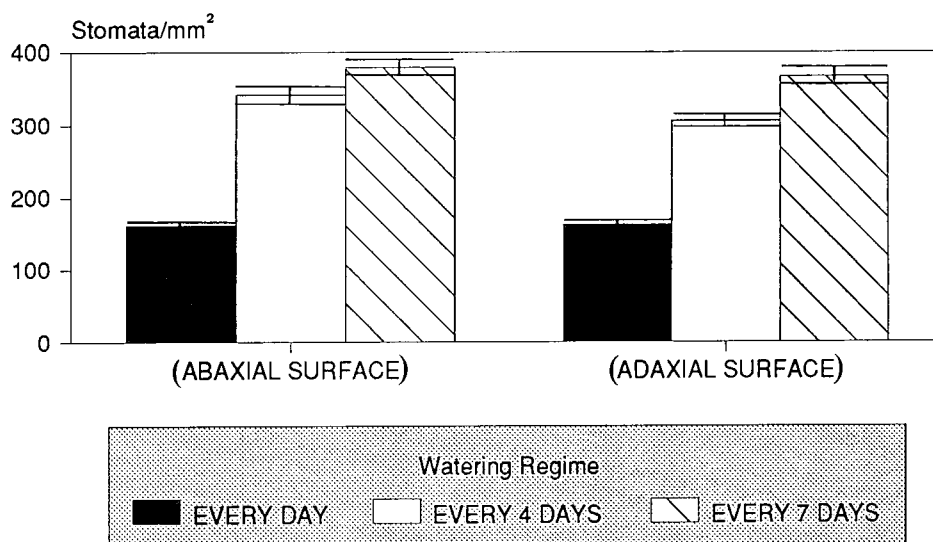


FIG. 32 **PLANTAGO LANCEOLATA L.**  
EFFECTS OF WATERING REGIMES  
ON THE STOMATAL INDEX



Site: Blackhall Rocks

FIG. 33 **PLANTAGO LANCEOLATA L.**  
EFFECTS OF WATERING REGIMES  
ON STOMATAL NUMBERS



Site: Blackhall Rocks

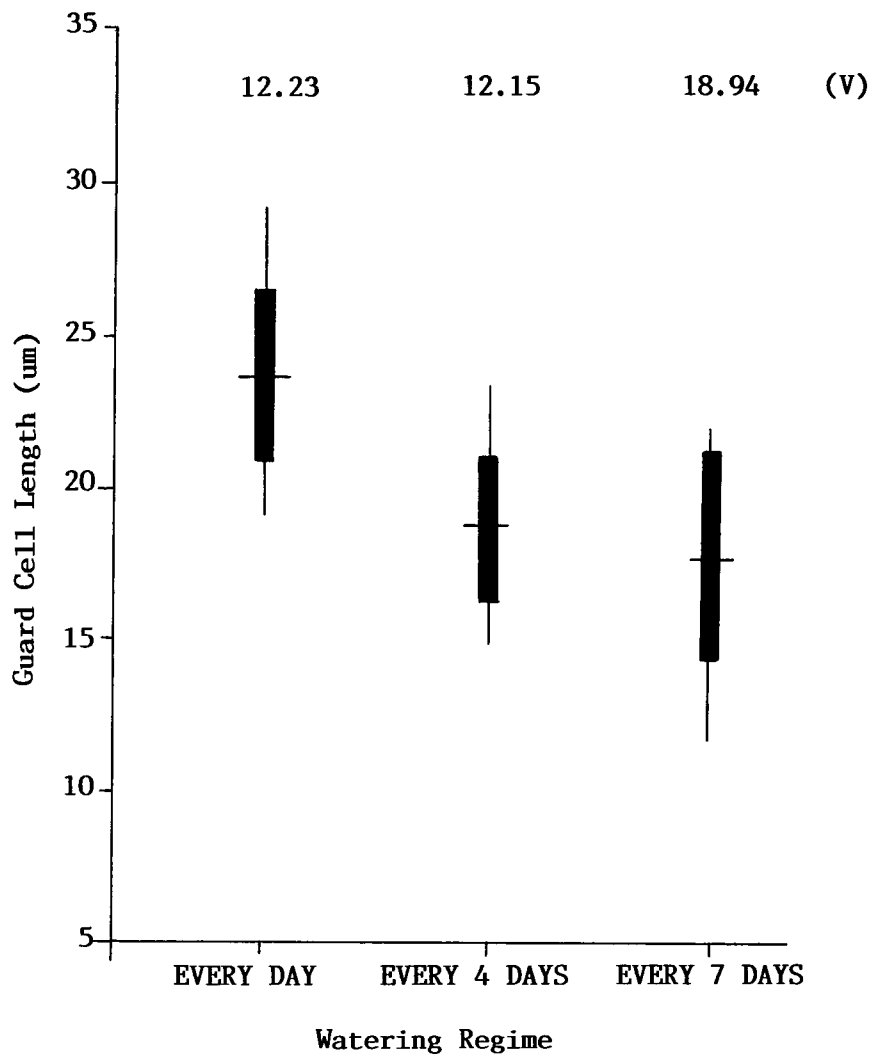
**FIG. 34** Mean Guard Cell Length in the abaxial surface of leaves of *P. lanceolata* L. taken from plants subjected to different watering regimes. Plants were from a lowland, coastal population (Blackhall Rocks). (P 0.0001) ANOVA.

The Standard Deviation, Range and Coefficient of Variation (V) are shown. N (Sample Number) = measurements from 25 guard cells.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days.

FIG. 34 GUARD CELL LENGTH

Watering Regime Effects on

*Plantago lanceolata* L.

Site: Blackhall Rocks

B2 *P. lanceolata* L.  
(Widdybank Fell)

Leaf area (Table 11) decreased very significantly with increasing stress ( $P < 0.000$ ).

**TABLE 11** Mean Leaf Area of youngest leaves of *P. lanceolata* L. plants under differing watering regimes.

TREATMENT	Area (cm <sup>2</sup> ) $\pm$ SE
CONTROL	1.25 $\pm$ 0.11
EVERY 4 DAYS	1.36 $\pm$ 0.19
EVERY 7 DAYS	0.60 $\pm$ 0.05
	***
n = 5	

The leaf SI (Fig. 35) decreases very significantly with increasing water stress in the abaxial surface ( $P < 0.0001$ ) from 32.4%  $\pm$  0.5% in control leaves to 25.0%  $\pm$  0.4% in 7-day water stressed leaves, with no significant difference occurring between the control and 4-day water stressed leaves (Scheffe). The small difference in the SI on the adaxial surface was not statistically significant ( $P=0.195$ ).

Fig. 36 shows that stomatal density increases very significantly ( $P < 0.0001$ ) with increasing water stress on both surfaces. A mean stomatal density of 344.1  $\pm$  8.1/mm<sup>2</sup> in controls leaves rose to 427.2  $\pm$  8.1/mm<sup>2</sup> under 4-day water stress treatment to 457.9  $\pm$  7.3/mm<sup>2</sup> under 7-day water stress treatment on the abaxial surface, differences being significant at the 5% level of probability between all treatments (Scheffe). No difference between 4 and 7-day treatments (375.2  $\pm$  16.9/mm<sup>2</sup> and 405.5  $\pm$  7.7/mm<sup>2</sup>) was found on the adaxial surface. Mean guard cell length (Fig. 37) decreased with increasing stress from 18.29  $\pm$  0.58  $\mu$ m to 14.70  $\pm$  0.65  $\mu$ m under 7-day stress treatment ( $P < 0.001$ ) (App. 1 ). The range of lengths is greater in leaves under 7-day water stress. The SD and Coefficient of Variation (23.27%) in size was higher in those under 7-day stress treatment.

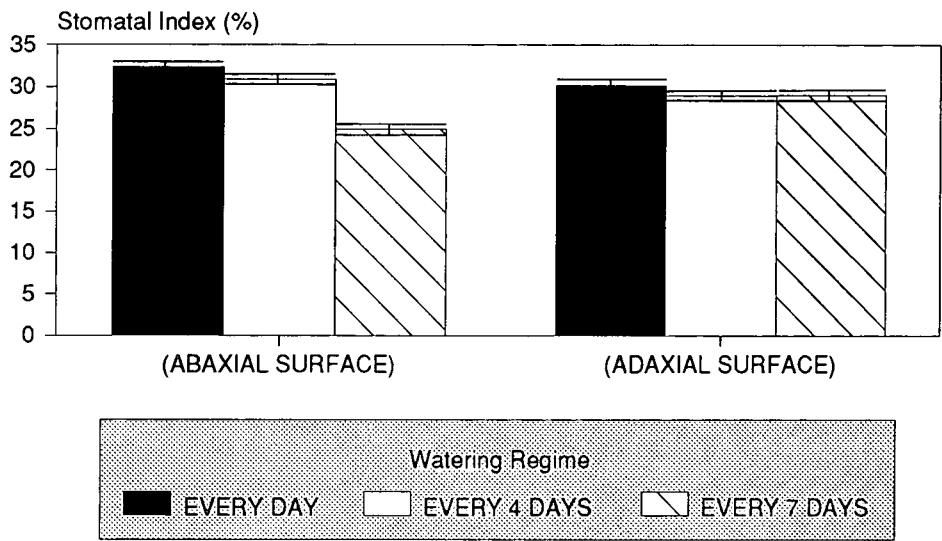
**FIG. 35**      Mean Stomatal Index in the adaxial and abaxial surface of leaves of *P. lanceolata* L. subjected to different watering regimes. (Widdybank Fell). ( $P < 0.0001$ ) ANOVA.

**FIG. 36**      Mean Stomatal Numbers/mm<sup>2</sup> in the adaxial and abaxial surface of leaves of *P. lanceolata* L. subjected to different watering regimes. (Widdybank Fell). ( $P < 0.0001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (sample number) = counts from 25 fields of view.

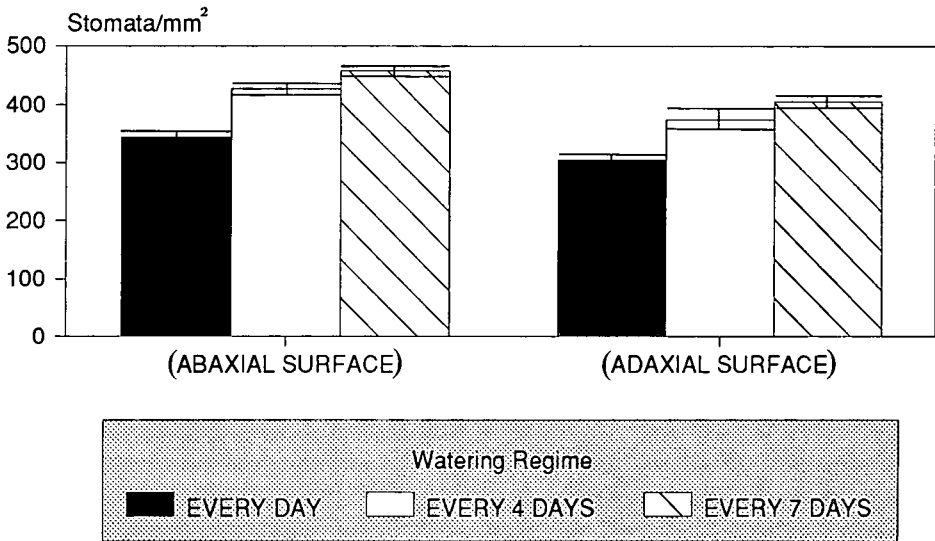
TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days

FIG. 35 **PLANTAGO LANCEOLATA L.**  
EFFECTS OF WATERING REGIMES  
ON THE STOMATAL INDEX



Site: Widdybank Fell

FIG. 36 **PLANTAGO LANCEOLATA L.**  
EFFECTS OF WATERING REGIMES  
ON STOMATAL NUMBERS



Site: Widdybank Fell

**FIG. 37**      Mean Guard Cell Length in the abaxial surface  
of leaves of *P. lanceolata* L. taken from  
plants subjected to different watering  
regimes. Plants were from an upland  
population (Widdybank Fell).  
( $P = 0.0001$ ) ANOVA.

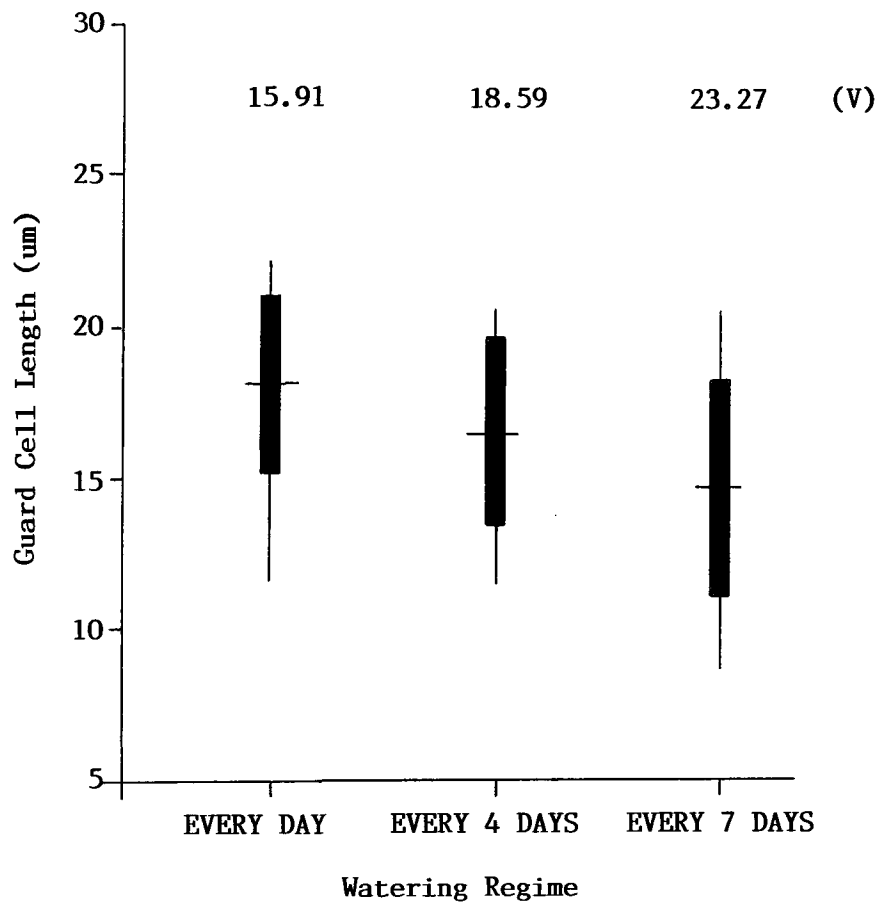
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days.

FIG. 37

## G U A R D   C E L L   L E N G T H

Watering Regime Effects on

*Plantago lanceolata* L.

Site: Widdybank Fell



**FIG. 38**      Mean Stomatal Index in leaves of  
*A. pseudoplatanus* L. seedlings subjected to  
different watering regimes. ( $P < 0.001$ ) ANOVA.

**FIG. 39**      Mean Stomatal Numbers/mm<sup>2</sup> in leaves of  
*A. pseudoplatanus* L. seedlings subjected to  
different watering regimes. ( $P < 0.001$ ) ANOVA.

In both Figures, error bars are indicated by vertical lines. N (Sample number) = counts from 25 half fields of view.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days.

FIG. 38 **ACER PSEUDOPLATANUS L.**  
EFFECTS OF WATERING REGIMES  
ON THE STOMATAL INDEX

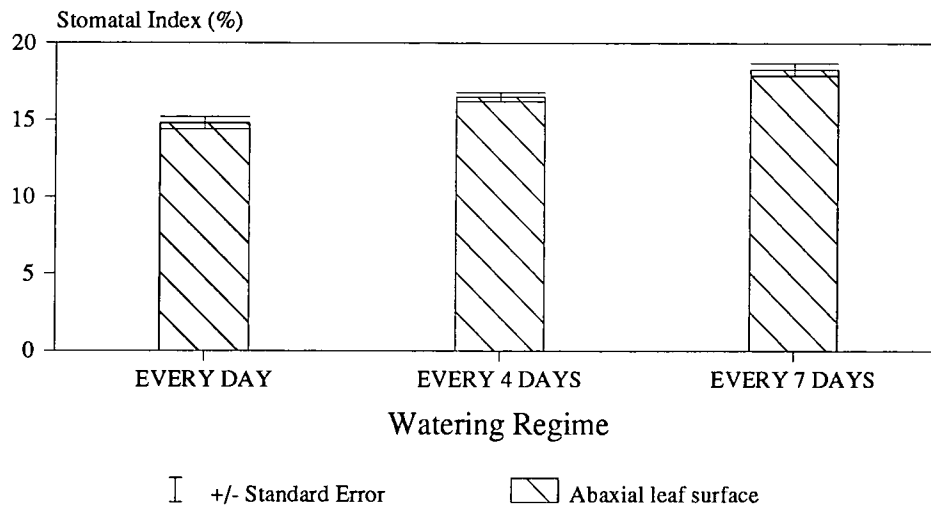
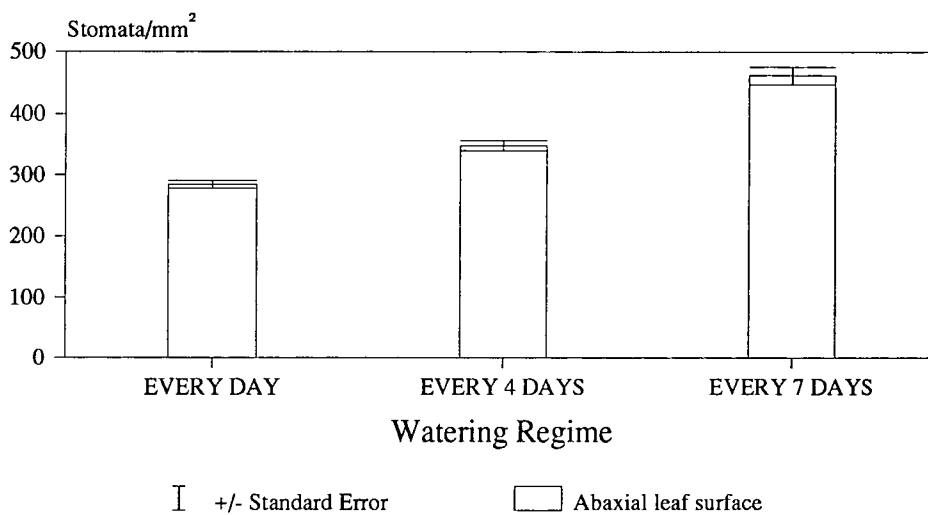


FIG. 39 **ACER PSEUDOPLATANUS L.**  
EFFECTS OF WATERING REGIMES  
ON STOMATAL NUMBERS



**FIG. 40** Mean Guard Cell Length in leaves of  
*A. pseudoplatanus* L. seedlings subjected to  
different watering regimes. ( $P < 0.001$ )ANOVA.

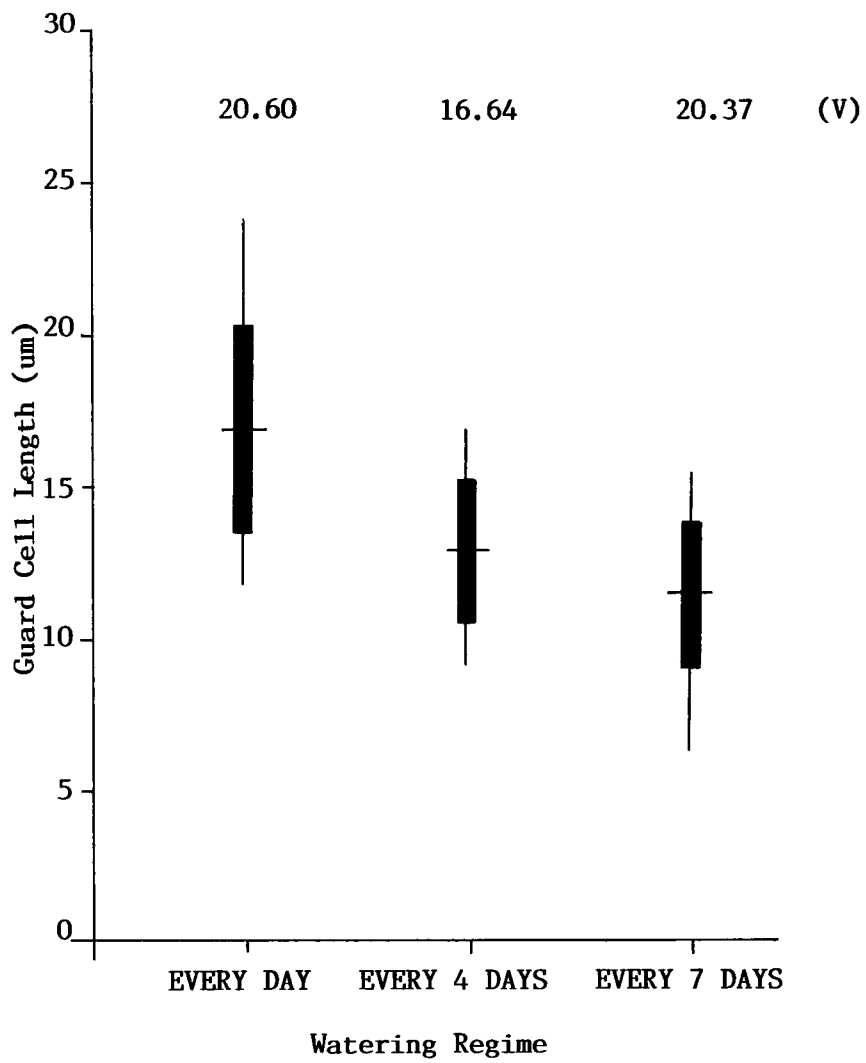
The Standard Deviation, Range and Coefficient of Variation (V) are shown. N  
(Sample Number) = measurements from 25 guard cells.

TREATMENT: Control	= Watered daily
4 days	= Watered every 4 days
7 days	= Watered every 7 days.

FIG. 40

## G U A R D   C E L L   L E N G T H

Watering Regime Effects on

*Acer pseudoplatanus* L.

C. *A. pseudoplatanus* L.

Leaf area of *A. pseudoplatanus* L. decreased significantly ( $P < 0.001$ ) with increasing water stress (Table 12), the differences occurring between each treatment ( $P < 0.05$ ). (Scheffe).

**TABLE 12** Mean Leaf Area of youngest leaves of *A. pseudoplatanus* L. seedlings under differing watering regimes.

TREATMENT	Area (cm <sup>2</sup> ) $\pm$ SE
CONTROL	69.61 $\pm$ 1.92
EVERY 4 DAYS	32.85 $\pm$ 3.82
EVERY 7 DAYS	21.36 $\pm$ 1.53
	***
n = 5	

Figs. 38 and 39 show that water stress increased both the leaf SI and number of stomata/mm<sup>2</sup> significantly ( $P < 0.001$ ). An increase in the mean leaf SI from 14.8%  $\pm$  0.4% in controls to 16.5%  $\pm$  0.3% under 4-day water stress treatment to 18.3%  $\pm$  0.4% under a 7-day water stress treatment was observed. The number of stomata/mm<sup>2</sup> rose from 283.9  $\pm$  6.40/mm<sup>2</sup> in control leaves to almost double (461.5  $\pm$  13.8/mm<sup>2</sup> in leaves under 7-day water stress treatment (App. 1). Differences in both stomatal parameters lay in the control leaves which varied significantly ( $P < 0.05$ ) from those under 4-day and 7-day stress treatments. (Scheffe). Guard cell length decreased significantly ( $P < 0.001$ ) with increasing stress (Fig. 40).

### 3.3 Chlorophyll Content

The results of the chlorophyll analyses are shown in Tables 13a. and 13b.

*S. caerulea* L. The total chlorophyll (chl) per unit fresh weight increased significantly ( $P = 0.002$ ) with increasing altitude in leaves collected from 4 study sites (Table 13a). Blackhall Rocks (0.781  $\pm$  0.089 mg g<sup>-1</sup>) compared with Widdybank Fell (1.700  $\pm$  0.089 mg g<sup>-1</sup>). Both chl a and chl b increased significantly ( $P < 0.002$ ) per unit fresh weight with increasing altitude. The ratio of chl a:chl b decreased significantly ( $P = 0.014$ ) with increasing altitude from 2.1  $\pm$  0.14 at Blackhall Rocks to 1.4  $\pm$  0.09 at Widdybank Fell.

**TABLE 13a.**  
**MEAN CHLOROPHYLL CONTENT** (mg/g/fr.wt) ( $\pm$  Standard Error)

<u>FIELD DATA - <i>SESLERIA CAERULEA</i> L.</u>				
SITE	TOTAL	CHLA	CHLB	RATIO a:b
BLACKHALL	0.781 $\pm$ .089	0.526 $\pm$ .064	0.255 $\pm$ .027	2.1 $\pm$ .14
PITTINGTON	1.208 $\pm$ .215	0.936 $\pm$ .034	0.517 $\pm$ .004	1.8 $\pm$ .10
LOW FORCE	1.491 $\pm$ .185	0.920 $\pm$ .092	0.577 $\pm$ .103	1.7 $\pm$ .15
WIDDYBANK	1.700 $\pm$ .089	0.989 $\pm$ .052	0.700 $\pm$ .061	1.4 $\pm$ .09
	**	**	**	**
(n=5 except Pittington n=3)				
<u>FIELD DATA - <i>PLANTAGO LANCEOLATA</i> L.</u>				
BLACKHALL	1.738 $\pm$ .093	1.096 $\pm$ .045	0.642 $\pm$ .055	1.7 $\pm$ .10
PITTINGTON S	2.689 $\pm$ .096	1.183 $\pm$ .005	1.506 $\pm$ .097	0.8 $\pm$ .06
PITTINGTON E	1.865 $\pm$ .142	1.097 $\pm$ .042	0.771 $\pm$ .105	1.5 $\pm$ .12
LOW FORCE	2.116 $\pm$ .012	1.109 $\pm$ .017	1.008 $\pm$ .027	1.1 $\pm$ .06
GARRIGIL L	0.982 $\pm$ .198	0.646 $\pm$ .120	0.347 $\pm$ .076	1.9 $\pm$ .10
GARRIGIL S	1.013 $\pm$ .126	0.681 $\pm$ .074	0.340 $\pm$ .046	2.0 $\pm$ .10
LEADGATE	1.005 $\pm$ .088	0.667 $\pm$ .055	0.351 $\pm$ .052	2.0 $\pm$ .18
WIDDYBANK	1.451 $\pm$ .002	0.978 $\pm$ .001	0.474 $\pm$ .001	2.1 $\pm$ .00
	***	***	***	***
(n=5 except: Leadgate n=4; Widdybank n=3; Low Force n=3.				
<u>WATER STRESS - <i>PLANTAGO LANCEOLATA</i> L.</u>				
BLACKHALL ROCKS				
CONTROL	1.535 $\pm$ .038	0.997 $\pm$ .023	0.558 $\pm$ .03	1.7 $\pm$ .04
4 DAYS	1.206 $\pm$ .099	0.800 $\pm$ .048	0.406 $\pm$ .052	2.0 $\pm$ .12
7 DAYS	1.554 $\pm$ .035	0.940 $\pm$ .008	0.615 $\pm$ .026	1.5 $\pm$ .06
(n=5)				

**TABLE 13b.**  
**MEAN CHLOROPHYLL CONTENT** (mg/g/fr.wt) ( $\pm$  Standard Error)

<u>FIELD DATA - ACER PSEUDOPLATANUS L.</u>				
SITE	TOTAL	CHLA	CHLB	RATIO a:b
FRANKLAND	1.537 $\pm$ .199	0.924 $\pm$ .099	0.613 $\pm$ .102	1.6 $\pm$ .12
LOW FORCE	1.589 $\pm$ .151	0.949 $\pm$ .066	0.641 $\pm$ .085	1.6 $\pm$ .12
HARTSIDE	1.747 $\pm$ .221	0.989 $\pm$ .068	0.758 $\pm$ .158	1.5 $\pm$ .20
(n=5)	NS	NS	NS	NS
<u>TRANSPLANTS - ACER PSEUDOPLATANUS L.</u>				
GILESGATE	1.895 $\pm$ .144	1.120 $\pm$ .043	0.776 $\pm$ .105	1.5 $\pm$ .18
ESH	1.763 $\pm$ .249	1.022 $\pm$ .095	0.742 $\pm$ .161	1.6 $\pm$ .22
HARTSIDE	1.521 $\pm$ .201	0.952 $\pm$ .077	0.569 $\pm$ .130	1.9 $\pm$ .20
(n=5)	NS	NS	NS	NS
<u>SHADING - ACER PSEUDOPLATANUS L.</u>				
CONTROL	2.008 $\pm$ .124	1.163 $\pm$ .017	0.846 $\pm$ .109	1.5 $\pm$ .16
40% SHADE	2.717 $\pm$ .059	1.182 $\pm$ .002	1.536 $\pm$ .060	0.8 $\pm$ .02
60% SHADE	2.890 $\pm$ .104	1.173 $\pm$ .007	1.173 $\pm$ .111	0.7 $\pm$ .05
(n=5)	***	NS	***	NS
<u>WATER STRESS - ACER PSEUDOPLATANUS L.</u>				
CONTROL	0.942 $\pm$ .108	0.648 $\pm$ .064	0.291 $\pm$ .043	2.3 $\pm$ .17
4 DAYS	1.185 $\pm$ .183	0.771 $\pm$ .110	0.415 $\pm$ .074	1.9 $\pm$ .09
7 DAYS	1.177 $\pm$ .041	0.773 $\pm$ .026	0.404 $\pm$ .020	1.9 $\pm$ .06
(n=5)				

**TABLE 13c.**  
**CHLOROPHYLL CONTENT PER UNIT AREA OF LEAF SURFACE IN**  
***A. PSEUDOPLATANUS L.* UNDER SHADE**

	AREA OF EACH LEAF (CM <sup>2</sup> )					MEAN (CM <sup>2</sup> )	CHL/ MG/M <sup>-2</sup>
CONTROL	39.10	35.66	32.35	46.00	34.12	37.45	<b>725</b>
40% SHADE	42.21	42.50	49.75	47.60	41.60	44.73	<b>646</b>
60% SHADE	49.50	50.00	49.30	58.50	46.25	50.71	<b>396</b>

*P. lanceolata* L. Table 13a shows considerable variation in the total chl content per unit fresh weight in leaves collected from *P. lanceolata* L. plants from eight populations of different altitude. Results show a generally higher level in plants up to 275m (Low Force) with levels falling at higher altitudinal sites. This same pattern is observed in the chl a and chl b content, with lower levels at the higher altitudinal sites. The ratio of chl a:chl b increases with increasing altitude from  $1.7 \pm 0.10$  to  $2.1$ . (Table 13a). Results were all highly significantly different as tested using ANOVA ( $P < 0.001$ ). Under water stress no pattern emerges both total chl, chl a and chl b varying between treatments, except under 4-day stress all levels are slightly lower, but the ratio is higher  $2.0 \pm 0.12$ .

*A. pseudoplatanus* L. Total chlorophyll per unit fresh weight increases with increasing altitude (Frankland -  $1.537 \pm 0.199$  mg g<sup>-1</sup>, Hartside  $1.747 \pm 0.221$  mg g<sup>-1</sup>) (Table 13b). chl a and chl b levels are similar between sites; rising only slightly at higher altitudinal sites; the ratio of chl a:chl b falls with altitude  $1.6 \pm 0.12$  to  $1.5 \pm 0.20$ . The Results were not statistically significant ( $P > 0.05$ ).

Transplants - *A. pseudoplatanus* L. Total chlorophyll per unit fresh weight (Table 13b) decreases with increasing altitude (Gilesgate Transplants -  $1.895 \pm 0.144$  mg g<sup>-1</sup>; and Hartside  $1.521 \pm 0.201$  mg g<sup>-1</sup>). Both chl a and chl b per unit fresh weight decrease with increasing altitude. The chl a:chl b ratio increases from  $1.5 \pm 0.18$  to  $1.9 \pm 0.20$  with increasing altitude. ANOVA showed results were not statistically significant ( $P > 0.05$ ).

Under water stress total chl per unit fresh weight in *A. pseudoplatanus* L. increases from  $0.942 \pm 0.108$  mg g<sup>-1</sup> in control leaves to  $1.177 \pm 0.041$  mg g<sup>-1</sup> under 7-day stress treatment.

Shading - *A. pseudoplatanus* L. Table 13b shows full grown low light leaves (60% shade) had significantly more ( $P < 0.001$ ) total chlorophyll per unit fresh weight ( $2.890 \pm 0.104$  mg g<sup>-1</sup>) than full grown high-light leaves ( $2.008 \pm 0.124$  mg g<sup>-1</sup>). Little variation in chl a content is shown, which was not significant ( $P = 0.454$ ); the level of chl b per unit fresh weight was significantly ( $P < 0.001$ ) higher in leaves under 40% shade ( $1.536 \pm 0.060$  mg g<sup>-1</sup>) than in leaves of the controls or under 60% shade. The chl a:chl b ratio decreases with increasing shade ( $1.5 \pm 0.16$  to  $0.7 \pm 0.05$  mg g<sup>-1</sup>). This result was not statistically significant ( $P = 0.458$ ).

Chlorophyll content per unit area of leaf surface was lower in the low-light leaves (396 mg m<sup>-2</sup>), than in high-light leaves (725 mg m<sup>-2</sup>).



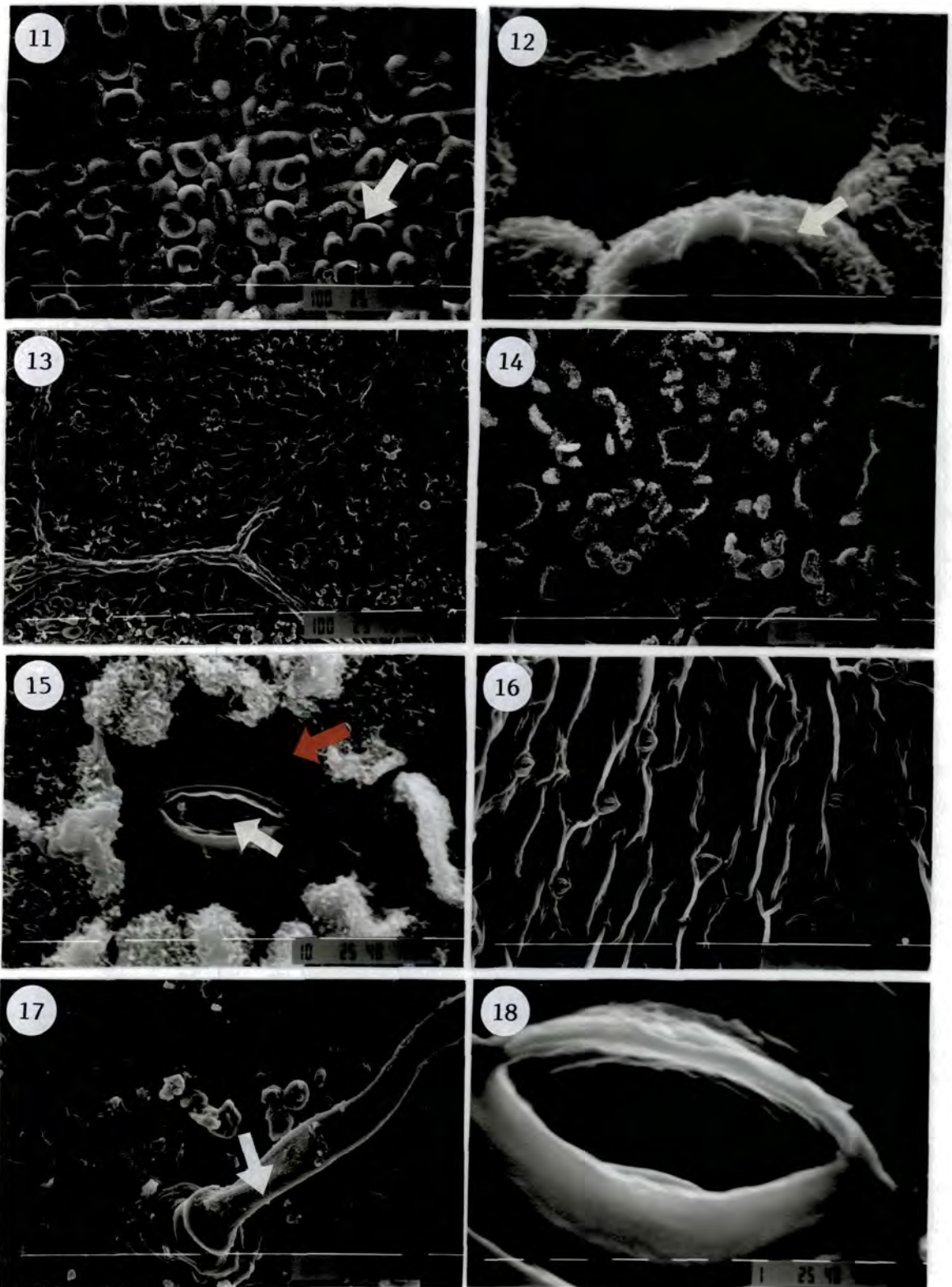
### **Scanning Electron Microscopy (SEM) of stomata**

**Scale:** The white horizontal lines on the SEM photographs opposite are equivalent to the first number in the bottom right-hand corner of each photograph (ie. 1, 10 or 100 um respectively). Twenty-five is the voltage, 48 is the working distance in millimetres and 1001 is a batch code.

**PLATES 11 and 12.** An SEM of the leaf surface of *S. caerulea* L. taken from a plant from Widdybank Fell, watered every seventh day. The deeply sunken pores (white arrow) are clearly visible on the adaxial surface (Plate 11). Plate 12 shows a stoma at a higher magnification. Both the pore, guard cells and subsidiary cells (white arrow) can be seen. The pore appears as a long slit.

**PLATES 13, 14 and 15.** Numerous stomata on the abaxial leaf surface of *A. pseudoplatanus* L. are shown in Plate 13. The leaf is transversed by numerous veins. The stomata (Plate 14) of *A. pseudoplatanus* L. under 40% shade are open wide and the guard cells clearly visible. The subsidiary cells surround the slightly sunken stomata. At high magnification (Plate 15) the spongy mesophyll cells are visible through the stoma, as this was the underside of the leaf, (white arrow). The edge of the guard cell is apparent (red arrow). Pores are distinctly oval in comparison to the grass.

**PLATES 16, 17 and 18.** Plate 16 shows stomata on the abaxial leaf surface of *P. lanceolata* L. from Widdybank Fell. The stomata are distributed randomly, and the guard cells slightly raised above the level of the other epidermal cells. Plate 17 was taken from the adaxial leaf surface. Comparing Plates 17 and 18, there are far more stomata on the lower than upper leaf surface. The adaxial surface was highly pubescent, as is shown by the leaf hair, (white arrow) highly magnified (Plate 17). A stoma of *P. lanceolata* L., highly magnified is shown in Plate 18. The palisade layer is just visible through the adaxial crescent-shaped stomatal pore.



### **Light and Fluorescent Microscopy of Stomata**

**Plates 19 - 25** are LMgraphs and plate 26 is a fluorescent microscope photograph.

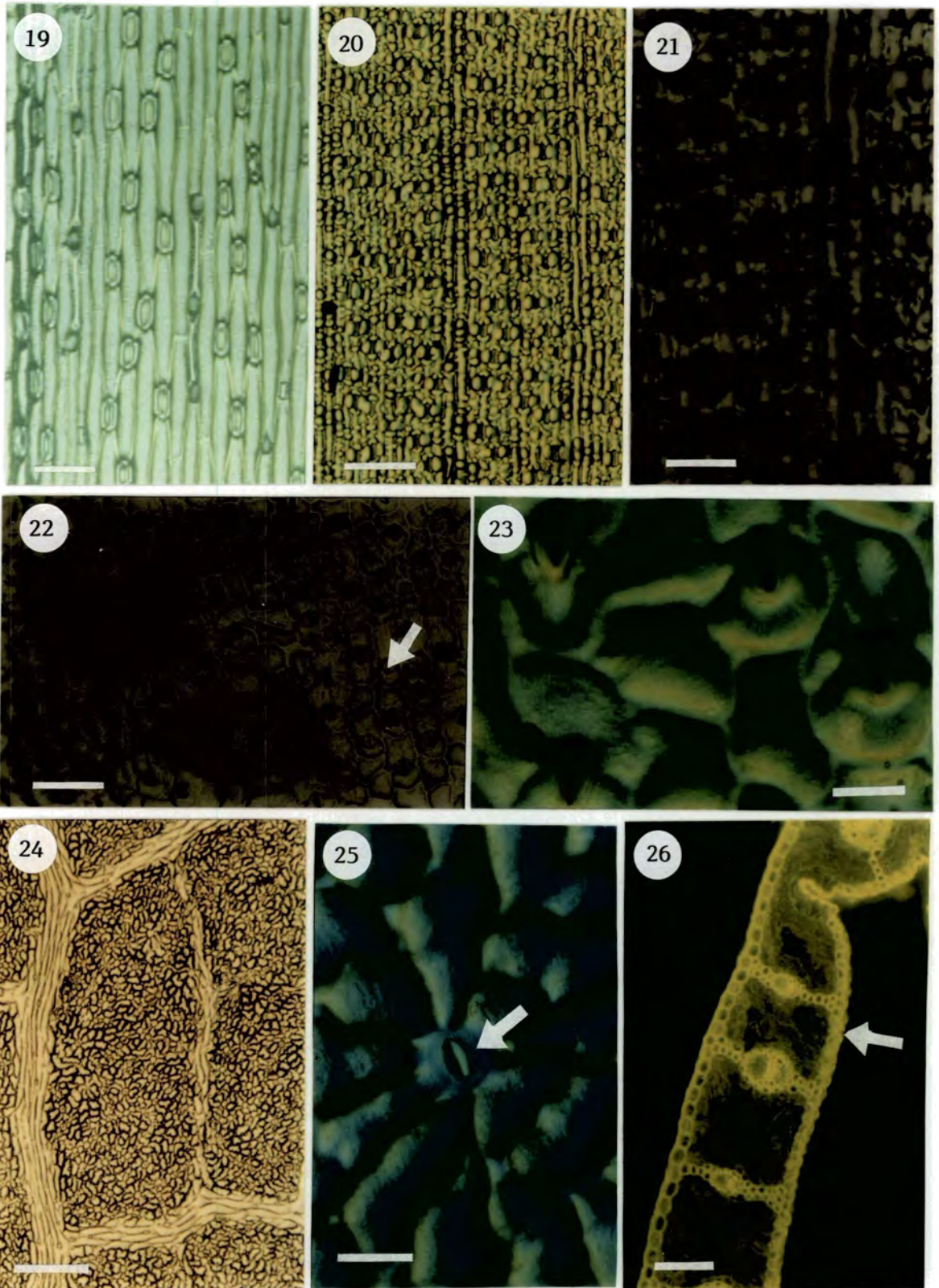
**Plates 19, 20, and 21.** - *S. caerulea* L. Plate 19 (scale bar, 50 um) shows the adaxial surface of *S. caerulea* L. from Blackhall Rocks. The stoma are arranged in parallel rows along the length of the leaf surrounded by two dumbbell shaped guard cells and distinctly long epidermal cells. Plate 20 (scale bar, 100 um), at a lower magnification, show numerous stomata on an adaxial leaf surface taken from Widdybank. Use of phase contrast enhances the surface cells. Plate 21 (scale bar, 40 um) shows the very sinuous appearance of the epidermal cells apparant on the leaves from higher altitudes and illustrates the difficulty in counting them.

**Plate 22 and 23.** Plate 22 (scale bar, 100 um) shows the random distribution of distinct stomata on an abaxial leaf surface from a *P. lanceolata* L. plant from Pittington Hill. The epidermal cells have characteristic undulate, anticlinal walls. This leaf was watered every fourth day and possible drought stress is shown by the number of stomata that are closed (white arrow). Plate 23, (scale bar, 20 um) at a higher magnification shows the epidermal cells are irregular in shape compared to the grass.

**Plate 24 and 25.** The abaxial leaf surface of *A. pseudoplatanus* L. from a tree at Hartside shows the presence of numerous stomata at low magnification (white arrow) and their random distribution. (Plate 24, scale bar, 100 um). Plate 25 (scale bar, 20 um) shows clearly under phase contrast one crescent-shaped stoma of this species surrounded by two guard cells (white arrow) and several subsidiary/epidermal cells.

**Plate 26** A transverse (TS) section of *S. caerulea* L. stained with auramine, fluorescing yellow in a blue light. A stoma (cut through its centre) is just visible on the adaxial leaf surface (white arrow), surrounded by two guard cells. Vascular bundles are also apparant. (Scale bar, 40 um).





## CHAPTER 4

### DISCUSSION

#### 4.1 Field Studies

Results from this study show how difficult it is to generalise how plants will respond to a changing environment. There is a differential response in different ecotypes, and the variation in stomatal parameters within a species is considerable.

Woodward (1983) has shown for a number of related and unrelated herbaceous species that plants from upland regions have lower specific leaf area (SLA) than lowland species. A similar pattern was found here in *S. caerulea* L. and *P. lanceolata* L. in the field which could be due to the environment as air temperature and wind speed decrease and increase with altitude respectively (See App. 3). Plants will develop a leaf better adapted to withstand higher wind-speeds correlated with a change in temperature.

Variability in the stomatal index (SI) and stomatal density with altitude has been reported by several investigators (Korner & Cochrane, 1981; Korner & Mayr, 1981; Korner, Bannister & Mark, 1986; Watkins, 1988 and Woodward, 1987). Korner & Cochrane (1985) reported the number of stomata per unit leaf area in *Eucalyptus pauciflora* L. to increase significantly up an elevational transect and the density was exactly the same on either side of a single leaf. Woodward (1986) found the SI in *Vaccinium myrtillus* L. increased with altitude on the adaxial surface.

In the present 'field' study, fluctuations with altitude occurred in *S. caerulea* L. for both stomatal parameters, but in general leaf stomatal density increased linearly with altitude except at Pittington Hill where high leaf stomatal density may be due to the very dry, shallow soil and exposed nature of the habitat. Restricted cell expansion may mean more stomata are packed together in a smaller unit area. Soil depth and moisture together explain 71% of the variation. This value is probably high due to covariance of these variables. West (1975) showed, in the populations he studied, a significant correlation between the size of the *S. caerulea* L. plants and soil depth at any one altitude. In this study the plants from dry habitats had smaller leaves with greater stomatal density than those collected from soils with higher moisture content. Comparing stomatal indices, the high proportion of stomata in Blackhall plants may be an adaptation to a harsher coastal environment. Humidity will be high and previous reports suggest the SI is lowered under high humidity (Salisbury, 1928); however, Blackhall is a very exposed site in general and combinations of factors such as temperature, irradiance or wind speed could override any one major determining factor. Salt spray or the salinity of the substrate could affect

the plant's metabolism. As the soil was fairly deep and flushed, this suggests the plant is able to replenish water lost by transpiration.

Both Pittington and Widdybank *S. caerulea* L. plants have similar SI's and density, yet morphologically they are very different; at Widdybank the plants were considerably smaller adopting a prostrate habit. Climatically the two regions differ, wind speed being much higher and mean temperatures lower at Widdybank (App. 3). Two separate ecotypes clearly exist. The Low Force plants have a fairly low leaf SI and stomatal density, even though they were growing out of a rock, under harsher conditions. One might have expected a high stomatal density. However, humidity would have been high at the site as they were close to water which may have resulted in a more mesic aerial environment with the production of fewer stomata.

In *P. lanceolata* L. a general increase in the leaf SI and stomatal density was seen with altitude, except for slightly higher values again at Blackhall. Leadgate and Widdybank plants also had similar SI's although an altitudinal difference of nearly 200m separated them. The stomatal density at Widdybank was high with much greater density on the abaxial surface and the plants were very small (see Plates 16 and 17). However, the soil was deeper at Leadgate and moisture content was higher which may account for the difference in the stomatal density. Statistical analysis suggests stomatal density appears to be higher where soil depth is shallow. Soil depth and moisture content explained 33.78% of the variation in the data. Penfound (1931) reported that plants growing in soil of high water content differed from those grown in dry soil by possessing more xylem, larger water conducting vessels and stomata were larger but less numerous.

*P. lanceolata* L. at Low Force had a low stomatal density, again this could be an adaptation to high humidity, soil moisture content and/or greater soil depth. The plants were also shaded by trees. In woodland the boundary layer is thinner than in open grassland and the temperature of the canopy close to air temperature so stomata have more control over transpiration (Woodward, 1989). Both depth of soil and altitude could be indirectly affecting guard cell length. Plants growing on deep soil tend to have larger leaf guard cells in most cases.

Within habitat, at two sites: Garrigil and Pittington, no difference in the SI's in *P. lanceolata* L. could be found and this appears to support Salisbury's (1927) original hypothesis suggesting differential growth of the epidermal cells. Both populations within habitat were different in morphology suggesting they were separate ecotypes. However, higher stomatal density on the abaxial leaf surface of plants on the stony soil at Garrigil and those under exposure at Pittington, suggests stomatal density is higher in this species where conditions are

more severe. This shows different characters of the phenotype can show varying degrees of plasticity. A study of Sea Plantain (*P. maritima* L.) by Watkins (1988) in North East England, showed considerable variation in leaf SI's between coastal and upland ecotypes. The plants showed phenotypic plasticity to the environment.

A linear increase in both the leaf stomatal density and SI with altitude in *A. pseudoplatanus* L. was interesting and the results suggest the former could be related to soil depth: as soil depth increases stomata density increases. However, further studies would need to be carried out to verify this. A general altitudinal effect related to CO<sub>2</sub> and partial pressures could have some effect as the SI and altitude were highly correlated. Frankland, a low altitudinal site, had a lower leaf stomatal index. Soil moisture content was fairly high at all three sites and this agrees with the habitat preference of *A. pseudoplatanus* L. Greater wind speeds at Low Force and Hartside may have influenced anatomical development.

Differences in guard cell length in all species generally showed an inverse correlation with stomatal frequency, particularly for *A. pseudoplatanus* L. This has also been found in other species (Stocker, 1960). In this study plants on very dry soil, especially *S. caerulea* L. and *P. lanceolata* L. tended to have smaller guard cells. Soil depth is suggested as a good predictor of guard cell length in all species which could account for the differences in size. Under optimum moisture conditions, the plants of both species were slightly larger suggesting the plants may have better root growth, enabling exploitation of the soil for translocation of nutrients and water uptake and subsequent growth. Root/shoot ratios would need to be examined.

Studies by Lewis (1969) have suggested that plants of more open habitats with harsher climatic conditions have higher SI's and small leaves combined with a higher stomatal index have more control over gas exchange and water loss. A high SI may mean photosynthesis can still occur in these plants at a level which maintains their growth despite the conditions.

*A. pseudoplatanus* L. seedlings showed considerable intraspecific variation in leaf SI's and stomatal density with both parameters increasing with increasing of leaf height up the stem. This highlights the importance of sampling from similar positions in each plant. As guard cell length decreases with height in the seedling, it appears the first leaves produce larger but fewer stomata. This suggests stomata production and size change with leaf insertion level in *A. pseudoplatanus* L. as has been reported in other species. (See Tichá, 1982 for a review).

## 4.2 Transplants

Some of the most extensive transplant experiments on ecotypes were carried out by Clausen, Kech and Hiesey (1939) over a 200-mile transect across Central California, on different species eg. *Potentilla glandulosa* L. Turesson (1925) maintained adaptation to the environment was sometimes by plastic responses, but more frequently it had a genetic base.

In the present study transplanted *A. pseudoplatanus* L. seedlings changed their SI's and stomatal density in their new environment, showing phenotypic plasticity dependent upon altitude of positioning. Both the SI and stomatal density increased with altitude. Leaf area was reduced with altitude. Comparison of stomatal parameters with measurements in leaves from mature trees in the field, shows lower stomatal parameters in the field samples, suggesting as the tree ages fewer stomata are produced. Alternatively, shading by other leaves may reduce both stomatal density and the proportion of stomata produced on subsequent leaves. Van Tienderen *et al.*, (1991), working with reciprocal transplants of *P. lanceolata* L. found the phenotype highly plastic, as exemplified by large differences between sites (early-mown, wet and late-mown hayfield and pasture) for seed survival, set, yield and incidence of flowering.

The *A. pseudoplatanus* L. seedlings in the present study were from the same province, therefore one would expect them to be genetically identical. Results suggest variation may be influenced by both climatic and microhabitat differences. Leaves from the Esh and Hartside seedling transplants showed considerable weathering. New leaves were smaller in size correlating with the higher stomatal density and indices. Perhaps harsher climatic conditions (high wind speed, lower temperatures, higher rainfall or a decrease in the partial pressure of CO<sub>2</sub>) affected their anatomical development. The transplants at Gilesgate were in a more sheltered position. A genotype-environment interaction is suggested as clearly these seedlings showed plasticity in response to the environment.

## 4.3 Experimental Studies

Numerous studies have looked at the effect of irradiance on leaf morphology and stomatal parameters (see Friend & Pomeroy, 1970; Gay & Hurd, 1975; Knecht & O'Leary, 1972; Kubinova, 1991; Solarova & Pospisilova, 1988; Wild & Wolf, 1980; and Wilson, 1966).

Leaf area increased with increasing shade in *A. pseudoplatanus* L. suggesting increased cell expansion. This supports results from other studies eg. *Zea mays* L. (Boyer, 1968). Other species have been affected by both cell division and expansion (see Friend & Pomeroy, 1970). In Cucumber (Wilson, 1966), leaves expanded more rapidly and had larger areas under low light



and also when the apex was removed than when present. Other factors such as light quality, temperature, or leaf water potential may be implicated. The R/FR ratio may directly affect stomatal production and leaf growth: in dark grown plants reducing radiation may stimulate, while FR may inhibit leaf expansion (Dale & Murray, 1968; Mitchell & Woodward, 1988; and Schoch *et al.*, 1987). Schoch (1977) found the SI of *Vigna sinensis* L. depended during stomatal differentiation on the level of active phytochrome in the plant at the beginning of the dark period. In the present study, since there was no difference in R/FR between the treatments under the shading frame, this suggests the quality of light had no effect. However, the ratio was considerably higher than for the daylight value of 1:1.

Fluence rate of incident radiation dramatically affects growth and development of leaves, often dicotyledons are larger than those grown at lower fluence rates. Cell expansion makes a significant contribution to leave size and for prolonged expansion to occur, there must be irreversible extension of the cell wall. Increased rates of leaf expansion have been found to correlate with increased wall extensibility in leaves of *Betula* (Taylor and Davies, 1985, 1988).

Usually, where drought and irradiance influence stomatal density, they do so by affecting the expansion of leaf area, causing the stomata to be packed more densely (drought) or less densely (low irradiance) through changes in the size of the epidermal cells (Tichá, 1982). Thus generally, when effects of epidermal cell size are taken into account in the stomatal index, changes largely disappear, indicating that the response simply involves the control of epidermal cell expansion (Woodward, 1987).

The results of a decrease in stomatal density and leaf SI in *A. pseudoplatanus* L. under shade support those of Schoch (1972) for *C. annuum* and Schoch *et al.*, (1977) for *V. sinensis* L. suggesting shade increases cellular expansion, but decreases cell division with the result that the number of cells in the shade were always fewer but larger than those in the sun. Clearly 14.8% of the epidermal cells form stomata under high light as compared with 18.3% for leaves under 60% shade. Stomatal production is directly affected. Again the difference in the length of the guard cells (larger under shade) could be due to increased cell expansion. In citrus species (Bahgat 1923 thesis unpub, in Hirano 1931), shading reduced the density of stomata and increased their size. Higher stomatal density in leaves grown under higher irradiance has also been reported in *Atriplex* (Björkman *et al.*, 1972); *Ficus* (Fails, Lewis & Barden, 1982); *Glycine max* (Ciha & Brun, 1975); *Helianthus annuus*, (Penfound, 1931) and *Iris* (Pazourek, 1970). Lower stomatal frequency has been reported in high light leaves too (Lichtenthaler *et al.*, 1981). Results of this study with *A. pseudoplatanus* L. show high light promotes stomatal differentiation.

Studies in France have shown that differences may be due to the leaf sensitivity to changes in light supply especially during unfolding of the blade. Schoch *et al.*, (1987) found it possible to affect leaf SI's of individual leaves on the same plant in different ways, suggesting differentiation of stomata in a leaf is a process specific to that leaf although light perception controlling differentiation probably takes places in other leaves (mature leaves). This suggests stomatal morphogenesis is under a genetic programme which can be affected by factors such as light energy, especially during the critical time preceding unfolding of the blade. Thus density could change but not of necessity if SI at late developmental stages.

Effects of water deficits on leaf and cell development have been studied by several investigators (see Clough & Milthorpe, 1975; Husain & Aspinall, 1970; Randall & Sinclair, 1988; Terry, Waldron & Olrich, 1971; Turner & Begg, 1981; Tyree & Karamanos (1980) and Yegappan *et al.* 1982). Development of water deficits leads to a wide range of responses by plants (Hsiao, 1973) affecting photosynthesis, respiration, absorption of water and mineral elements, growth and development, nitrogen metabolism and reproduction. It is generally accepted that a reduction in cell growth is one of the most discernible effects of water deficits.

The responses of the three species in this study to different watering regimes show considerable variation. Both the populations of *S. caerulea* L. from Blackhall and Widdybank show an increase in stomatal density and a decrease in the leaf SI with a reduction in water. However, despite these effects both maintain ecotypical variation, ie, the Blackhall population still has a higher mean SI and lower stomatal density compared to the Widdybank Population after water was withheld for periods of 7 days. This illustrates they are genetically distinct populations, that are expressing phenotypic plasticity under varying watering regimes.

The Blackhall population had a significant variation in leaf area, decreasing with increasing stress, suggesting plasticity to be greater in these plants because of their origin in a more mesic environment. The reduction in surface/volume ratio would be an adaptation to conserve water by reducing their transpiring surface. It appears natural selection operates to produce genotypes that are most efficient at gathering the resources of local habitats.

No significant difference was found in the leaf area of the *S. caerulea* L. plants from Widdybank (Table 9) following treatment, suggesting that the plants are already at the stress stage of reduced growth and added water stress does not bring about further reduction in leaf area ie. they are more phenotypically constant. SEM of the leaves watered every seventh day showed deeply sunken pores which could be an adaptation to a harsher environment (Plate 11).

In a grass new cells emerge from the meristem situated at the base of the leaf. It appears that as soon as the leaf blade meristem is subject to a different but constant watering regime the rate of initiation of stomata changes, perhaps to compensate for the change in water supply. Studies in *Helianthus annuus* L. showed water stress increased stomata density, but reduced stomata sizes and area so that the area of the stomata apparatus per unit leaf area or the stomata number per leaf remained unchanged (Rawson & Craven, 1980).

Seyed-Yagoobi (1977) found *S. caerulea* L. plants increased the number of stomata per unit area under water stress. If cell size of any species (mesophyte or xerophyte) is reduced by loss of turgor during expansion, it seems logical that these leaves will have a higher stomatal density than those grown in the absence of water stress, if the number of potential guard cells is unaffected. It is probable plants growing in drier soil possess a more efficient absorbing system relative to their transpiring surface.

An increase in stomatal density with water stress may be the result of restricted leaf expansion, the decrease in the production of stomata could be result of inhibition of stomatal mother cell differentiation. Schurman (1959) reported decreases in the stomata:epidermal cell ratio by withholding water. Ciha & Brun (1975) found field grown Soybeans had significantly greater stomatal density and smaller leaf area than non-water stressed plants.

Stomatal density increases and leaf area is reduced in both *P. lanceolata* L. populations, suggesting this species may be more sensitive than *S. caerulea* L. to varying watering regimes in the field. The SI is reduced on both surfaces in the Blackhall population and only on the abaxial surface in the Widdybank population. Relative stomatal density and indices remain close to the results from the field populations in each case, suggesting, as in *S. caerulea* L., each population is a distinct ecotype. No reduction in the adaxial leaf SI at Widdybank could be due to the leaf pubescence on the adaxial surface which may have been a specific ecotypic adaptation as protection to the plant to reduce water loss at a higher altitude.

In this study, *A. pseudoplatanus* L. responded differently to the varying watering regime. Both stomatal density and the leaf SI increased with increasing water stress and leaf area was also reduced suggesting cell expansion was affected but cell division was not. Clearly stomatal production has been promoted; which suggests the plant is not directly subjected to 'water stress' effects. The imposed watering regime may not be 'stressful enough' or perhaps is indirectly affecting other aspects of the plant's physiology ie. hormone levels which may stimulate or enhance stomatal production. Some evidence suggests differences in stomatal response to water stress are partially determined by genetic differences in the capacity to produce ABA (see

Quarrie 1981). The general cell biochemistry may also be disturbed. Fluctuations in temperature and variations in humidity in the greenhouse may have affected stomatal production. The difficulty in counting the epidermal cells on some of the *A. pseudoplatanus* L. leaves may have led to experimental error, suggesting care is needed in interpretation of the SI for this species. In all three species, guard cell size decreased with increasing water stress.

The duration of water stress and time of application are important. Probably over the duration of the experiment the seedlings were coping with the deficit by reducing stomatal pore size on both young and mature leaves to control transpiration. Transpiration measurements would need to be carried out to see if differences occurred. Muchow & Sinclair (1989) found epidermal conductance increased with increasing stomatal density in *Sorghum bicolor* L.

This study shows that the ratio of stomata to epidermal cells does not remain constant under varying watering regimes in either of these three species. Plant species may vary in their ability to maintain solute potential in expanding cells; in some cases influx of water may dilute the cell contents whereas under water stress, other species may lower the solute potential of growing cells by secreting solutes into the vacuole, leading to increased movement of water into the cells, increased cell turgor and maintained cell expansion. This latter phenomenon could contribute to the higher SI in *A. pseudoplatanus* L.

#### 4.4 Chlorophyll

Chlorophyll (chl) content, which can influence the rate of photosynthesis in plants, is known to vary between species and also within species. Studies in Gymnosperms have shown an increase in the chl a and chl b per fresh and dry matter with increasing altitude, and a decrease in the chl a chl b ratio. This is inconsistent with the literature on Angiosperms. (Morales *et al.*, 1952).

Positive correlations have been found between the content of chl and dry matter production (Tieszen and Johnson, 1968) or photosynthetic (PS) rate (Šesták, 1966), suggesting possible use of chl as a productivity parameter. A number of authors (see Tranquillini, 1964) suggest lack of CO<sub>2</sub> may be an important factor in the reduction of PS at high altitudes. If true it could be expected that plants growing at high altitudes would evolve more efficient carbon-fixing mechanisms than those growing at low altitudes and would fix larger quantities of carbon dioxide at relatively low concentrations of [CO<sub>2</sub>]<sub>air</sub>.

Results of this preliminary study showed in *A. pseudoplatanus* L. and *S. caerulea* L. that an increase in the total chl content is correlated with an increase in the stomatal density with

altitude. The 'efficiency' of stomatal pores as pathways for gaseous diffusion depends essentially on pore size and distribution in the epidermis (Teare, Peterson & Law, 1971). The greater stomatal density in xeromorphic leaves (eg. at Widdybank) may facilitate the entry of CO<sub>2</sub> by decreasing the diffusive resistance to CO<sub>2</sub> uptake and accelerating photosynthesis since even though the stomatal openings are smaller the rate of gas diffusion does not decrease proportionately with the reduction in aggregate pore area. This suggests the plants are photosynthesising more efficiently.

Morphological differences particularly in *P. lanceolata* L. suggested differences in physiology might exist. Where stomatal density and chlorophyll are high at reduced CO<sub>2</sub> tensions, this suggests the plants may be fairly efficient and able to maintain a positive carbon balance. However, total chlorophyll content in *P. lanceolata* L. does vary between sites. This could be influenced by the lower sample size for some sites, variable leaf morphology, or the fact that chl was measured on a fresh weight basis. Chl content is generally high where stomatal density is high (See Blackhall, Pittington E and Widdybank), the exceptions are populations from both Low Force and Pittington (S) which had low and moderate stomatal density and very high total chl values (2.689 mg g<sup>-1</sup> and 2.116 mg g<sup>-1</sup>) respectively. The high chl levels could be the result of the fairly shaded habitats at these two locations as shade, in the present study increased total chl levels in *A. pseudoplatanus* L. Macro and microclimatic differences, ie. humidity at Low Force, temperature and exposure at Pittington (S) could have increased the total chl levels. Both populations appear to be ecotypes with a different genetic make-up.

A higher chl content suggests a greater proportion of incident light is absorbed. This could also be important at higher altitudes where cloud cover and growing season is shorter which may restrict photosynthesis. It seems likely that high altitude plants need to maximise photosynthesis in a shorter time compared to coastal/lowland plants; this they do by having increased chl levels so they can build reserves. The difference in chl content between lowland and upland sites could also be due to plant chloroplast and cell size besides differing environmental conditions at the site.

Photosynthetic rates are also affected by temperature, water conditions and light intensity. Increasing the chl content could be an adaptation by the plant to compensate for variations in environmental conditions so that the plants can still maintain a high enough photosynthetic rate to optimise carbon gain under various climatic conditions. A study of *P. maritima* L. by Watkins (1988) showed plants from inland sites were smaller, had higher chl levels and more stomata compared to coastal sites. Boonkerd (1980) found in *Primulas* both

positive and negative correlations between the PS rates (measured as O<sub>2</sub> evolution) and chl content, and O<sub>2</sub> evolution and stomatal density.

In this study transplanted seedlings of *A. pseudoplatanus* L. showed a decrease in total chl content, an increase in the chl a:b ratio but an increase in stomatal density with altitude suggesting chl production may be slow or that the component is broken down at higher altitudes, perhaps due to destruction of the reaction centres owing to harsher climatic conditions or the photosystems may be more sensitive to irradiance (See Björkman, 1968). It is possible that since the plants were all from the same province, the acclimation period was not long enough especially at the harsher habitats. The seedlings at high altitude may have reduced leaf absorptance and PS rates and may not need as much chl. Measurements of PS rates would need to be carried out to measure their efficiency.

Chl a:b ratios in plants vary depending upon nutritional status or other environmental growth conditions and may play an important role in the light response of photosynthesis. Chlorophyll levels change with more rapid destruction of chl a than chl b being common in Autumn leaves of tree species (Wolf, 1951). Chl concentration varies too with different CO<sub>2</sub> fixation cycles - plants with C4 cycle of CO<sub>2</sub> fixation had higher concentrations of chl a. (see Black and Maine 1970).

The light intensity under which a plant is grown can have important effects on PS capacity. It is well known that during growth light intensity as well as light quality affects a number of component processes of PS, leaf morphology and chloroplast ultrastructure (Wild & Wolf, 1980). Reports have shown that low-light chloroplasts are richer in chl than high-light chloroplasts and that chloroplasts from plants grown at low light intensities have better grana development and the volume of stroma relative to chloroplast volume appears smaller (Ballantine and Forde, 1970).

In the present study, leaves of *A. pseudoplatanus* L. grown at low light intensity had more chl per unit fresh weight than at high intensities and the a:b ratio decreased as light intensity was lowered, supporting the results of Rhizopoulou *et al.*, (1991) for evergreen sclerophylls. Chloroplasts from the low light leaves had more chl than the controls. Perhaps at high irradiance some of the chl is destroyed by photooxidation; a study of chloroplast morphology would infer if changes had taken place. However, per unit area of leaf chl levels do decrease with decreasing light intensity from 725 mg/m<sup>-2</sup> in controls to 396 mg/m<sup>-2</sup> under 60% shade, (Table 13c). These plants also had a lower stomatal density in shade suggesting that overall photosynthesis may be operating at a slower rate. Results measured in the present study also support those of Clough,

Teeri & Alberte, (1979) who reported high irradiance produces less chl per fresh matter, but a higher amount of chl per surface unit.

Under varying watering regimes, there was a differential response by the two species studied. *A. pseudoplatanus* L. had increased chl levels per mg fr. wt. with increasing stress and the density of stomata increased in this species. This is interesting, but could mean chloroplast replication is unaffected as is cell division, again perhaps because the plant is not feeling direct 'water stress effects'; the irradiance gradient may be overcoming the harsh watering regime as the plants received adequate light.

In *P. lanceolata* L. no real pattern emerges: under 4-day stress the stomatal density has risen and chl levels decreased suggesting plants may be photosynthesising inefficiently. Under 7-day stress chl levels rose again, suggesting plants may have acclimated to the water shortage. Perhaps water stress may lead to a lower pigment synthesis and reduced hormone activity (Cytokinins) in some species. Heichel (1971) looked at maize varieties differing in stomatal density and PS capacity. The variety with the lesser stomatal density and higher total leaf resistance to water loss had faster net PS than the variety with greater stomatal density demonstrating mesophyll resistance. Recent studies by Quick *et al.* (1990) comparing four species showed that soil water depletion over a period of several days (4 to 7 days) may result in different effects on mesophyll conductance.

#### **4.5 Significance and interrelationships of the study**

This stomatal study shows intraspecific and interspecific variation occurs both in situ in the field and under manipulated environmental conditions in these three species suggesting that many environmental effects interact together in determining stomatal parameters. The results are commensurate with the early work of Turesson (1925, 1930) on ecotypes, differences in morphology and anatomy being correlated with habitat differences. This study has shown plant ecotypes respond differently in the extent of their phenotypic plasticity and suggests care should be taken when predicting a plant's general response to changing environmental conditions. It appears from this study that species along an environmental gradient can reveal evolutionary adaptive trends to particular stress conditions. Selection could act to increase the frequency of those genotypes with the highest fitness under a new environmental regime.

The experimental studies show that a small change in photon flux density can change the morphology and anatomy of a plant as can soil moisture. Through effects on net CO<sub>2</sub> exchange, previous studies have shown changes in leaf anatomy can affect both photosynthesis

and water use efficiency (ratio of PS to transpiration). In the light of current interest in increased CO<sub>2</sub> levels it would be useful to run experiments where both CO<sub>2</sub> levels and other environmental variables are interacting together as in the field situation. In this and other laboratory studies of stress physiology a single component of the environment was measured. In field studies, analysis is complicated by the fact that two or more factors may vary simultaneously as was seen in this study. Kramer (1981) has argued that increased atmospheric CO<sub>2</sub> concentrations will have least effect on growth of plants in conditions where light, water and nutrients are already limiting photosynthetic rate.

Preliminary results of chlorophyll levels shows the plasticity of the plant to respond to changing environmental conditions. A plant with high stomatal density and chlorophyll content suggests the plant is functioning efficiently. High stomatal density and low chlorophyll content suggests the plant is working under-efficiently or that it is adapted to the macro and micro-environmental conditions, perhaps through using more efficient CO<sub>2</sub> utilisation systems. Photosynthesis has been shown to increase with increasing CO<sub>2</sub> concentrations. Sharkey (1985) suggests increasing CO<sub>2</sub> concentration would shift the balance towards PS and away from photorespiration and would reduce nitrogen investment in Rubisco and improve water use efficiency during photorespiration. Long-term exposure to an atmosphere enriched with CO<sub>2</sub> has reportedly resulted in acclimation in rates of growth and PS and Rubisco activity in various species.

The stomatal studies of herbarium specimens (Woodward, 1987) indicate the proportion of stomata in many species was greater 200 years ago and there is a suggestion that photosynthetic rate of plants of the last centuries (when CO<sub>2</sub> levels were lower) may have been reduced indicating the likelihood of lower water use efficiencies than the present (Woodward, 1987). This implies that plants growing in the future may be more efficient in terms of WUE.

The variation in stomatal density has significance in plant breeding too. By crossing plants having high and low frequency of stomata it would be possible to obtain hybrids having different stomatal densities and thus to alter associated physiological processes. Also as stomatal resistance is related to photosynthesis and water use efficiency, stomatal density could be an important criterion to use in selecting parents for producing high yielding crop plants eg. wheat.



## CHAPTER 5

### CONCLUSION

In conclusion of this study, considerable variation occurs in both the leaf morphology, stomatal indices, stomatal density and guard cell length both within and between species. There is a differential response by ecotypes both in the field and under manipulated environmental conditions. Clearly the environmental conditions, soil depth, moisture content and altitude contribute to a high percentage of the stomatal variation in the data. A significant increase in the leaf SI and stomatal density with altitude was found in *A. pseudoplatanus* L. This pattern also occurred in the other two species, but with some variation across sites.

The results of this study strongly suggest the existence of a plastic response in all three species to varying watering regimes and in *A. pseudoplatanus* L. to light. From the chlorophyll analyses, the field results suggest stomatal density is correlated with an increase in chl levels in *A. pseudoplatanus* L. and in some of the samples of *S. caerulea* L and *P. lanceolata* L. Transplanted seedlings of *A. pseudoplatanus* L. appear not to have acclimated to the new conditions with regard to chl levels. Samples from water-stressed plants show a differential response in the two genera.

In the present study significant differences in leaf SI's both in the field and under experimental conditions, have shown altitude and other environmental parameters were not just affecting differential epidermal cell expansion as earlier hypothesised. Clearly both within and between species and genera plants respond differently to environmental conditions. Many apparent direct effects of environmental variables may act indirectly. One can reject the original hypothesis that the SI is due to differential growth of the epidermal cells, but accept that environmental variables may influence a differential direct or indirect plastic response by the plants to alter stomatal production and development.

Following this study it would be necessary to look at stomatal parameters, particularly the SI in other species from different areas, to see if variability is as high. Growing plants up from a seed source would be useful in a similar study. However, reexamination using the SI should be treated with caution as reliability of the index is consequent upon an accurate epidermal cell count which may be difficult in some species. The use of the SEM and image analysis for automatic measurements would give rapid results and greater resolution (see Fricker, Grantz & Willmer, 1991).

Chl levels could also be looked at further, perhaps at different times of the year. Examination of mesophyll anatomy in leaves (see Kubinova, 1991 for *Hordeum vulgare* L.), root to shoot ratios and measurements of PS and transpiration rates could complement this work. Work on the interaction of increased CO<sub>2</sub> levels in conjunction with manipulated environmental conditions is already being studied. (See Bhattachaya *et al.*, 1990). It would be interesting to look more closely at an interacting effect in these three species by manipulating several environmental variables at once under controlled conditions.

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## **APPENDICES**

**APPENDIX 1****Sesleria caerulea L.**

	<u>MEAN</u> (25 fields of view)	<u>+/-SE</u>	<u>STDS</u>
<b><u>Stomatal Index</u></b>			
Blackhall	0.330	0.008	0.038
Pittington	0.299	0.007	0.036
Low Force	0.263	0.005	0.026
Widdybank			
<b><u>Stomata/mm<sup>2</sup></u></b>			
Blackhall	107.212	6.649	13.247
Pittington	305.269	8.177	40.887
Low Force	220.972	5.685	28.326
Widdybank	308.542	4.787	23.934
<b><u>Guard Cell Length (um)</u></b>			
Blackhall	24.11	0.41	2.04
Pittington	18.05	0.49	2.46
Low Force	14.46	0.33	1.63
Widdybank	17.23	0.45	2.27

**Plantago lanceolata L.****Stomatal Index**

Blackhall	(B)	0.260	0.005	0.023
Blackhall	(T)	0.254	0.004	0.021
Pittington (E)	(B)	0.269	0.004	0.019
Pittington (E)	(T)	0.273	0.004	0.020
Pittington (S)	(B)	0.259	0.004	0.019
Pittington (S)	(T)	0.279	0.006	0.030
Low Force	(B)	0.266	0.005	0.025
Low Force	(T)	0.258	0.005	0.026
Garrigil (L)	(B)	0.255	0.006	0.029
Garrigil (L)	(T)	0.261	0.004	0.019
Garrigil (S)	(B)	0.266	0.004	0.020
Garrigil (S)	(T)	0.269	0.005	0.024
Leadgate	(B)	0.292	0.006	0.029
Leadgate	(T)	0.296	0.006	0.029
Widdybank	(B)	0.335	0.006	0.030
Widdybank	(T)	0.298	0.006	0.030

B = Bottom of leaf T = Top of leaf

Pittington (E) and Pittington (S) = Exposed and Shaded respectively.

Garrigil (S) and Garrigil (L) = Small and large leaves respectively.

<u>Cont</u>		<u>MEAN</u>	<u>+/-SE</u>	<u>STDS</u>
		(25 fields of view)		
<u><b>Stomata/mm<sup>2</sup></b></u>				
Blackhall	(B)	251.662	4.537	22.684
Blackhall	(T)	215.243	5.802	29.008
Pittington (E)	(B)	247.980	4.809	24.043
Pittington (E)	(T)	171.458	5.518	27.590
Pittington (S)	(B)	207.659	3.974	19.872
Pittington (S)	(T)	187.417	4.526	22.630
Low Force	(B)	171.458	4.586	22.929
Low Force	(T)	153.862	3.616	18.080
Garrigil (L)	(B)	203.785	4.572	22.860
Garrigil (L)	(T)	166.957	2.995	14.977
Garrigil (S)	(B)	245.524	4.537	22.684
Garrigil (S)	(T)	188.645	4.423	22.115
Leadgate	(B)	146.087	4.114	20.571
Leadgate	(T)	184.962	3.685	18.424
Widdybank	(B)	376.471	7.902	39.511
Widdybank	(T)	277.442	9.711	48.555
<u><b>Guard Cell Length (um)</b></u>				
Blackhall	(B)	21.29	0.50	2.48
Pittington (E)	(B)	22.11	0.61	3.07
Pittington (S)	(B)	22.52	0.33	1.63
Low Force	(B)	25.11	0.40	1.99
Garrigil (L)	(B)	20.70	0.44	2.20
Garrigil (S)	(B)	17.52	0.50	2.51
Leadgate	(B)	20.64	0.48	2.42
Widdybank	(B)	24.23	0.45	2.25
<u><b><i>Acer pseudoplatanus L.</i></b></u>		(25 half fields of view)		
<u><b>Stomatal Index</b></u>				
Franklands & Kepier		0.082	0.003	0.013
Low Force		0.091	0.003	0.016
Hartside		0.127	0.003	0.013
<u><b>Stomata/mm<sup>2</sup></b></u>				
Franklands & Kepier		110.477	4.870	24.351
Low Force		154.668	7.511	37.853
Hartside		161.214	5.056	25.278

<u>Cont</u>	<u>MEAN</u>	<u>+/-SE</u>	<u>STDS</u>
<u>Guard Cell Length (um)</u>	(25 half fields of view)		
Franklands & Kepier	22.17	0.90	4.51
Low Force	16.76	0.77	3.87
Hartside	16.58	0.71	3.56

*Acer pseudoplatanus L.*  
Transplants

<u>Stomatal Index</u>			
Gilesgate	0.136	0.003	0.016
Esh	0.142	0.003	0.013
Hartside	0.165	0.004	0.019
<u>Stomata/mm<sup>2</sup></u>			
Gilesgate	207.042	5.325	26.623
Esh	233.229	6.887	34.437
Hartside	280.693	5.967	29.835

Guard Cell Length (um)

Gilesgate	18.87	0.39	1.93
Esh	17.35	0.51	2.55
Hartside	14.88	0.50	2.52

WATER STRESS EXPERIMENTS

*Sesleria caerulea L.*  
(Blackhall Rocks)

	(25 fields of view)		
<u>Stomatal Index</u>			
Control	0.348	0.009	0.043
4 Days	0.311	0.006	0.029
7 Days	0.274	0.006	0.030
<u>Stomata/mm<sup>2</sup></u>			
Control	115.396	4.440	22.202
4 Days	195.601	4.549	22.745
7 Days	215.652	4.173	20.866

<u>Cont</u>	<u>MEAN</u> (25 fields of view)	<u>+/-SE</u>	<u>STDS</u>
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**Guard Cell Length (um)**

Control	22.93	0.50	2.51
4 Days	18.76	0.53	2.63
7 days	17.58	0.41	2.06

***Sesleria caerulea L.***  
**(Widdybank Fell)**

**Stomatal Index**

Control	0.308	0.006	0.029
4 Days	0.284	0.004	0.021
7 Days	0.268	0.006	0.028

**Stomata/mm<sup>2</sup>**

Control	292.942	6.078	30.388
4 Days	314.680	5.226	26.129
7 Days	349.054	11.381	56.907

**Guard Cell Length (um)**

Control	18.70	0.44	2.19
4 Days	16.82	0.50	2.51
7 Days	15.29	0.56	2.81

***Plantago lanceolata L.***  
**(Blackhall Rocks)**

**Stomatal Index**

Control	(B)	0.268	0.006	0.028
Control	(T)	0.281	0.007	0.035
4 Days	(B)	0.256	0.005	0.025
4 Days	(T)	0.256	0.005	0.026
7 Days	(B)	0.243	0.002	0.011
7 Days	(T)	0.257	0.006	0.029

<u>Cont</u>		<u>MEAN</u> (25 fields of view)	<u>+/-SE</u>	<u>STDS</u>
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**Stomata/mm<sup>2</sup>**

Control	(B)	161.637	3.444	17.220
Control	(T)	162.455	4.549	22.745
4 Days	(B)	342.097	10.892	54.461
4 Days	(T)	306.496	5.364	26.821
7 Days	(B)	379.744	10.421	52.107
7 Days	(T)	366.650	11.248	56.241

**Guard Cell Length (um)**

Control	(B)	23.87	0.58	2.92
4 Days	(B)	18.93	0.46	2.30
7 Days	(B)	17.58	0.67	3.33

***Plantago lanceolata L.*  
**(Widdybank Fell)******Stomatal Index**

Control	(B)	0.324	0.005	0.026
Control	(T)	0.302	0.006	0.031
4 Days	(B)	0.309	0.005	0.023
4 Days	(T)	0.290	0.004	0.021
7 Days	(B)	0.250	0.004	0.021
7 Days	(T)	0.291	0.003	0.017

**Stomata/mm<sup>2</sup>**

Control	(B)	344.143	8.160	40.801
Control	(T)	305.269	8.512	42.559
4 Days	(B)	427.212	8.126	40.630
4 Days	(T)	375.243	16.952	84.758
7 Days	(B)	457.903	7.336	36.682
7 Days	(T)	405.524	7.676	38.378

**Guard Cell Length (um)**

Control	(B)	18.29	0.58	2.91
4 Days	(B)	16.35	0.61	3.04
7 Days	(B)	14.70	0.68	3.42

<u>Cont</u>		<u>MEAN</u>	<u>+/-SE</u>	<u>STDS</u>
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*Acer pseudoplatanus L.*

(25 half  
fields of  
view)

Stomatal Index

Control	(B)	0.148	0.004	0.021
4 Days	(B)	0.165	0.003	0.017
7 Days	(B)	0.183	0.004	0.022

Stomata/mm<sup>2</sup>

Control	(B)	283.967	6.396	31.979
4 Days	(B)	347.798	8.352	41.761
7 Days	(B)	461.548	13.829	69.147

Guard Cell Length (um)

Control	(B)	16.87	0.70	3.48
4 Days	(B)	12.90	0.43	2.15
7 Days	(B)	11.46	0.47	2.33

*Acer pseudoplatanus L.*  
Seedling Variation

Stomatal Index

Bottom	Plant 1	0.102	0.004	0.019
Middle	Plant 1	0.134	0.003	0.015
Top	Plant 1	0.155	0.004	0.020
Bottom	Plant 2	0.099	0.004	0.020
Middle	Plant 2	0.128	0.004	0.019
Top	Plant 2	0.139	0.004	0.018
Bottom	Plant 3	0.097	0.005	0.023
Middle	Plant 3	0.134	0.004	0.020
Top	Plant 3	0.180	0.004	0.022
Bottom	Plant 4	0.113	0.006	0.031
Middle	Plant 4	0.142	0.005	0.025
Top	Plant 4	0.157	0.004	0.021

Stomata/mm<sup>2</sup>

Bottom	Plant 1	103.930	4.407	22.035
Middle	Plant 1	177.581	5.377	26.883
Top	Plant 1	278.238	10.229	51.147



<u>Cont</u>		<u>MEAN</u>	<u>+/-SE</u>	<u>STDS</u>
<u><i>Acer pseudoplatanus L</i></u>		(25 half fields of view)		
Bottom	Plant 2	102.293	5.149	25.743
Middle	Plant 2	171.035	6.009	30.045
Top	Plant 2	226.682	8.673	43.367
Bottom	Plant 3	101.475	5.345	26.727
Middle	Plant 3	174.308	6.483	32.413
Top	Plant 3	265.963	6.785	33.927
Bottom	Plant 4	124.389	7.274	36.368
Middle	Plant 4	180.036	7.376	36.882
Top	Plant 4	281.511	11.347	56.734

**Guard Cell Length (um)**  
(One seedling)

Bottom	Plant 1	19.93	0.56	2.82
Middle	Plant 1	19.17	0.57	2.86
Top	Plant 1	17.11	0.35	1.74

*Acer pseudoplatanus L.*  
**Shading Experiment**

**Stomatal Index**

Control	0.148	0.004	0.021
40% Shade	0.165	0.003	0.017
60% Shade	0.183	0.004	0.022

**Stomata/mm<sup>2</sup>**

Control	283.967	6.396	31.979
40% Shade	347.798	8.352	41.761
60% Shade	461.548	13.829	69.147

**Guard Cell Length (um)**

Control	17.11	0.74	3.72
40% Shade	17.00	0.56	2.79
60% Shade	18.01	0.48	2.40

**APPENDIX 2****STATISTICAL RESULTS OF MANOVA, ANOVA, ONE-WAY ANOVA, STEPWISE  
MULTIPLE REGRESSIONS AND T-TESTS.****FIELD DATA*****Sesleria caerulea* L.**

	<b><u>DF</u></b>	<b><u>F RATIO</u></b>	<b><u>SIGN OF F</u></b>
NUMBER	3,96	277.80	.0000
INDEX	3,96	19.44	.0000
GUARD	3,96	91.97	.0000
AREA	3,96	79.16	.0000

***Plantago lanceolata* L.**  
**(Bottom of leaf)**

NUMBER	7,192	193.84	.000
INDEX	7,192	29.39	.000
GUARD	7,192	24.90	.000
AREA	7,192	334.38	.000

**(Top of leaf)**

NUMBER	2,192	50.16	.000
INDEX	2,192	10.80	.000
GUARD	2,192	49.03	.000

***Acer pseudoplatanus* L.**

NUMBER	7,72	21.44	.000
INDEX	7,72	68.43	.000
GUARD	7,72	12.00	.000
AREA	7,72	17.75	.000

***Acer pseudoplatanus* L.**  
**Transplants**

NUMBER	2,72	38.56	.0000
INDEX	2,72	21.28	.0000
GUARD	2,72	18.43	.0000
AREA	2,72	21.38	.000

*Acer pseudoplatanus L.*  
Seedling Variation

UP SEEDLING

PLANT 1

NUMBER	2,72	150.14	.000
INDEX	2,72	59.14	.000
GUARD	2,72	8.34	.001

PLANT 2

NUMBER	2,72	84.49	.000
INDEX	2,72	28.70	.000

PLANT 3

NUMBER	2,72	176.561	.000
INDEX	2,72	85.66	.000

PLANT 4

NUMBER	2,72	80.66	.000
INDEX	2,72	18.10	.000

BETWEEN 4 SEEDLINGS

NUMBER	3,296	1.99	.116
INDEX	3,296	3.70	.012

*Sesleria caerulea L.*  
Water Stress  
(Blackhall Rocks)

NUMBER	2,72	145.90	.0000
INDEX	2,72	15.36	.0000
GUARD	2,72	25.84	.0000
AREA	2,72	7.74	.0009

Water Stress  
(Widdybank Fell)

NUMBER	2,72	12.64	.0000
INDEX	2,72	15.99	.0000
GUARD	2,72	11.50	.0000
AREA	2,72	1.41	.2512

*Plantago lanceolata* L.Water Stress (Blackhall Rocks)(Bottom of leaf)

NUMBER	2,72	171.63 .0000
INDEX	2,72	7.21 .0014
GUARD	2,72	33.08 .0000
AREA	2,72	20.97 .0000

(Top of leaf)

NUMBER	2,72	187.64 .000
INDEX	2,72	5.74 .000

Water Stress (Widdybank Fell)(Bottom of leaf)

NUMBER	2,72	55.80 .0000
INDEX	2,72	68.75 .0000
GUARD	2,72	8.34 .0006
AREA	2,72	58.20 .0000

(Top of leaf)

NUMBER	2,72	18.94 .000
INDEX	2,72	1.67 .195

*Acer pseudoplatanus* L.Water Stress

NUMBER	2,72	80.40 .000
INDEX	2,72	17.83 .000
GUARD	2,72	26.62 .000
AREA	2,72	554.89 .000

*Acer pseudoplatanus* L.Shading Stress

NUMBER	2,72	22.14 .0000
INDEX	2,72	21.14 .0000
GUARD	2,72	0.84 .4362
AREA	2,72	62.24 .0000

CHLOROPHYLL*Sesleria caerulea* L.

TOTAL	3,14	8.463 .002
CHLOROA	3,14	9.853 .001
CHLOROB	3,14	8.377 .002
A:B RATIO	3,14	5.091 .014

*P. lanceolata L.*

TOTAL	7,27	24.603 .000
CHLOROA	7,27	13.050 .000
CHLOROB	7,27	32.208 .000
A:B RATIO	7,27	20.231 .000

*A. pseudoplatanus L.*

TOTAL	2,12	.429 .661
CHLOROA	2,12	.259 .776
CHLOROB	2,12	.523 .606
A:B RATIO	2,12	.196 .824

*A. pseudoplatanus L - Transplants*

TOTAL	2,12	.879 .440
CHLOROA	2,12	1.273 .315
CHLOROB	2,12	.511 .613
A:B RATIO	2,12	3.035 .086

*A. pseudoplatanus L - Shading Stress*

TOTAL	2,12	22.161 .000
CHLOROA	2,12	.844 .454
CHLOROB	2,12	22.361 .000
A:B RATIO	2,12	.833 .458

**ANOVA ONE-WAY RANGE=SCHEFFE**  
**(\* Significant at the 0.05 % Level)**

**FIELD DATA**

**Sesleria caerulea L.**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	BLA	PIT	LOW	WDB
BLACKHALL	107.21				
PITTINGTON	305.27	*			
LOW FORCE	220.97	*	*		
WIDDYBANK	308.54	*	NS	*	

<u>INDEX (%)</u>	<u>MEAN</u>	BLA	PIT	LOW	WDB
BLACKHALL	33.00				
PITTINGTON	29.90	*			
LOW FORCE	26.30	*	*		
WIDDYBANK	30.20	*	NS	*	

<u>GUARD (UM)</u>	<u>MEAN</u>	BLA	PIT	LOW	WDB
BLACKHALL	24.11				
PITTINGTON	18.05	*			
LOW FORCE	14.46	*	*		
WIDDYBANK	17.23	*	NS	*	

<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>	BLA	PIT	LOW	WDB
BLACKHALL	1.38				
PITTINGTON	3.38	*			
LOW FORCE	0.96	NS	*		
WIDDYBANK	0.87	NS	*	NS	

**Plantago lanceolata L.**  
**(Bottom of leaf)**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	LEA	LOW	GAR (L)	PIT (S)	GAR (S)	PIT (E)	BLA	WDB
LEADGATE	146.10								
LOW FORCE	171.46								
GARRIGIL (L)	203.79	*	*						
PITTINGTON (S)	207.06	*	*						
GARRIGIL (S)	245.52	*	*	*	*				
PITTINGTON (E)	247.98	*	*	*	*				
BLACKHALL	251.68	*	*	*	*				
WIDDYBANK	376.47	*	*	*	*	*	*	*	

<u>INDEX (%)</u>	<u>MEAN</u>	GAR (L)	PIT (S)	BLA	LOW	GAR (S)	PIT (E)	LEA	WDB
GARRIGIL (L)	25.47								
PITTINGTON (S)	25.87								
BLACKHALL	26.04								
LOW FORCE	26.57								
GARRIGIL (S)	26.60								
PITTINGTON (E)	26.89								
LEADGATE	29.22	*	*	*	*				
WIDDYBANK	33.52	*	*	*	*	*	*	*	

<u>GUARD (UM)</u>	<u>MEAN</u>	GAR (S)	LEA	GAR (L)	BLA	PIT (E)	PIT (S)	WDB	LOW
GARRIGIL (S)	17.52								
LEADGATE	20.64	*							
GARRIGIL (L)	20.70	*							
BLACKHALL	21.29	*							
PITTINGTON (E)	22.11	*							
PITTINGTON (S)	22.52	*							
WIDDYBANK	24.23	*	*	*	*				
LOW FORCE	25.11	*	*	*	*	*	*		

<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>	WDB	GAR (S)	BLA	LEA	LOW	PIT (E)	GAR (L)	PIT (S)
WIDDYBANK	0.80								
GARRIGIL (S)	1.81	*							
BLACKHALL	2.10	*	*	*					
LEADGATE	3.25	*	*	*					
LOW FORCE	3.63	*	*	*					
PITTINGTON (E)	4.25	*	*	*	*				
GARRIGIL (L)	6.13	*	*	*	*	*	*		
PITTINGTON (S)	10.63	*	*	*	*	*	*	*	

**(Top of Leaf)**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	LOW	GAR (L)	LEA	PIT (S)	GAR (S)	PIT (E)	BLA	WDB
LOW FORCE	153.86								
GARRIGIL (L)	166.93								
LEADGATE	186.96	*							
PITTINGTON (S)	187.41	*							
GARRIGIL (S)	188.64	*							
PITTINGTON (E)	191.91	*							
BLACKHALL	215.24	*	*	*					
WIDDYBANK	277.44	*	*	*	*	*	*	*	



		BLA	LOW	GAR (L)	GAR (S)	PIT (E)	PIT (S)	LEA	WDB
<u>INDEX (%)</u>	<u>MEAN</u>								
BLACKHALL	25.39								
LOW FORCE	25.77								
GARRIGIL (L)	26.07								
GARRIGIL (S)	26.94								
PITTINGTON (E)	27.29								
PITTINGTON (S)	27.88								
LEADGATE	29.56	*	*	*					
WIDDYBANK	29.83	*	*	*	*				

*Acer pseudoplatanus L.*

		FRANKLAND	LOW FORCE	HARTSIDE
<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>			
FRANKLAND	110.48			
LOW FORCE	154.67	*		
HARTSIDE	161.22	*	NS	

		FRANKLAND	LOW FORCE	HARTSIDE
<u>INDEX (%)</u>	<u>MEAN</u>			
FRANKLAND	8.16			
LOW FORCE	9.11	NS		
HARTSIDE	12.68	*	*	

		FRANKLAND	LOW FORCE	HARTSIDE
<u>GUARD (UM)</u>	<u>MEAN</u>			
FRANKLAND	16.50			
LOW FORCE	16.75	NS		
HARTSIDE	22.16	*	*	

		FRANKLAND	LOW FORCE	HARTSIDE
<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>			
FRANKLAND	49.61			
LOW FORCE	51.60	NS		
HARTSIDE	58.13	*	*	



**Acer pseudoplatanus L.**  
**Transplants**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	GILESGATE	ESH	HARTSIDE
GILESGATE	207.04			
ESH	233.22	*		
HARTSIDE	281.52	*	*	
<u>INDEX (%)</u>	<u>MEAN</u>	GILESGATE	ESH	HARTSIDE
GILESGATE	13.16			
ESH	13.80	NS		
HARTSIDE	16.08	*	*	
<u>GUARD (UM)</u>	<u>MEAN</u>	GILESGATE	ESH	HARTSIDE
GILESGATE	18.87			
ESH	13.80			
HARTSIDE	16.08	*	*	
<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>	GILESGATE	ESH	HARTSIDE
GILESGATE	57.28			
ESH	52.80	*		
HARTSIDE	50.49	*	NS	

**Plantago lanceolata L. (Blackhall Rocks)**  
**Water Stress**  
**(Bottom of leaf)**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	161.64			
4-DAY	342.09	*		
7-DAY	379.74	*	*	
<u>INDEX (%)</u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	26.8			
4-DAY	25.6	NS		
7-DAY	24.3	*		

<u>GUARD (UM)</u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	23.87			
4-DAY	18.93	*		
7-DAY	17.58	*	NS	

		CONTROL	4-DAY	7-DAY
<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>			
CONTROL	4.60			
4-DAY	4.00	*		
7-DAY	3.32	*	*	

(Top of leaf)

		CONTROL	4-DAY	7-DAY
<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>			
CONTROL	162.45			
4-DAY	306.50	*		
7-DAY	379.74	*	*	

<u>INDEX (%)</u>	<u>MEAN</u>			
CONTROL	28.10			
4-DAY	25.60	*		
7-DAY	25.60	*	NS	

*Plantago lanceolata* L. (Widdybank Fell)Water Stress(Bottom of leaf)

		CONTROL	4-DAY	7-DAY
<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>			
CONTROL	344.14			
4-DAY	427.21	*		
7-DAY	487.90	*	*	

		CONTROL	4-DAY	7-DAY
<u>INDEX (%)</u>	<u>MEAN</u>			
CONTROL	32.4			
4-DAY	30.9	NS		
7-DAY	25.0	*	*	

<u>GUARD (UM)</u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	18.29			
4-DAY	16.35	NS		
7-DAY	14.70	*	NS	

<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	1.25			
4-DAY	1.36	NS		
7-DAY	0.60	*	*	

(Top of leaf)

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	305.26			
4-DAYS	375.24	*		
7-DAYS	405.52	*	NS	

INDEX (%)

No significant difference between  
2 groups.

*Acer pseudoplatanus L.*  
Water Stress

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	283.97			
4-DAY	347.80	*		
7-DAY	461.85	*	NS	

<u>INDEX (%)</u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	14.8			
4-DAY	16.5	*		
7-DAY	18.3	*	NS	

<u>GUARD (UM)</u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	16.87			
4-DAY	12.90	*		
7-DAY	11.45	*	NS	
<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>			
CONTROL	69.61			
4-DAY	32.85	*		
7-DAY	21.36	*	*	

**Sesleria caerulea L. (Blackhall Rocks)**  
**Water Stress**

		CONTROL	4-DAY	7-DAY
<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>			
CONTROL	115.40			
4-DAY	195.60	*		
7-DAY	215.64	*	*	
<u>INDEX (%)</u>	<u>MEAN</u>			
CONTROL	33.36			
4-DAY	30.68	NS		
7-DAY	26.96	*	*	
<u>GUARD (UM)</u>	<u>MEAN</u>			
CONTROL	22.93			
4-DAY	19.12	*		
7-DAY	17.58	*	NS	
<u>AREA (CM<sup>2</sup>)</u>	<u>MEAN</u>			
CONTROL	1.61			
4-DAY	1.42	NS		
7-DAY	1.31	*	NS	

**Sesleria caerulea L. (Widdybank Fell)****Water Stress**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	CONTROL	4-DAY	7-DAY
CONTROL	292.99			
4-DAY	314.68	NS		
7-DAY	349.05	*	*	

**INDEX (%)**

CONTROL	30.40	
4-DAY	27.96	*
7-DAY	26.32	*NS

**GUARD (UM)**

CONTROL	18.70	
4-DAY	16.82	*
7-DAY	15.29	*NS

**AREA (CM<sup>2</sup>)** No significant differences.

**Acer pseudoplatanus L.****Shading Experiment**

<u>STOMATA/MM<sup>2</sup></u>	<u>MEAN</u>	CONTROL	40% SHADE	60% SHADE
CONTROL	299.96			
40% SHADE	189.86	*		
60% SHADE	166.948	*	NS	

**INDEX (%)**

<u>INDEX (%)</u>	<u>MEAN</u>			
CONTROL	16.4			
40% SHADE	13.2	*		
60% SHADE	13.0	*	NS	

**GUARD (UM)**

<u>GUARD (UM)</u>	<u>MEAN</u>			
CONTROL	37.45			
40% SHADE	44.73	None significantly different		
60% SHADE	50.71			

**AREA (CM<sup>2</sup>)**

CONTROL	37.45			
40% SHADE	44.73	*		
60% SHADE	50.71	*	*	

**T-TESTS**

Statistical Tests of significance for Stomatal indices and Stomatal density in *P. lanceolata* L.

**Lower versus upper surface**

<b><u>SITE</u></b>	<b><u>INDEX</u></b>	<b><u>STOMATA/MM<sup>2</sup></u></b>
BLACKHALL	NS	***
PITTINGTON E	NS	***
PITTINGTON S	**	**
LOW FORCE	NS	**
LEADGATE	NS	***
GARRIGIL S	NS	***
GARRIGIL L	NS	***
WIDDYBANK	***	***

Multiple Linear Regressions  
Summary Output

S. caerulea L.

Stomatal Frequency

1. Soil Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of</u>	<u>Mean</u>
			<u>Squares</u>	<u>Square</u>
Multiple R	.74996			
R Square	.56245			
Ad R Square	.55798	Regression 1	421301.79	21301.79
Standard Error	57.83069	Residual 98	327750.07	3344.38
		F = 125.972	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	-33.80	3.01	-.75	-11.22	.0000
Constant	294.64	7.82		37.66	.0000

2. Soil Moisture

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of</u>	<u>Mean</u>
			<u>Squares</u>	<u>Square</u>
Multiple R	.84809			
R Square	.71926			
Ad R Square	.71347	Regression 2	538762.34	69381.17
Standard Error	46.56107	Residual 97	210289.51	2167.93
		F = 124.257	sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	-70.70	5.57	-1.57	-12.70	.0000
Soil Moist	3.60	.49	.91	7.361	.0000
Constant	260.93	7.79		33.50	.0000

3. Altitude

		<u>Analysis of Variances</u>		
		<u>DF</u>	<u>Sum of</u>	<u>Mean</u>
			<u>Squares</u>	<u>Square</u>
Multiple R	.94695			
R Square	.89671			
Ad R Square	.89348	Regression 3	671678.95	23892.98
Standard Error	28.38957	Residual 96	77372.91	805.97
		F = 277.793	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	-227.07	12.64	-5.04.	-17.96	.0000
Soil Moisture	14.18	.87	3.58	16.19	.0000
Altitude	-.74	.06	-1.63	-12.84	.0000

Index1. Soil Moist

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.58134			
R Square	.33796			
Ad R Square	.33120	Regression 1	.05013	.05013
Standard Error	.03166	Residual 98	.09821	.00100
		F = 50.027	Sign F =	.0000

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Moisture	.001	1.449	.58	7.07	.0000
Constant	.270	.005		53.37	.0000

Guard1. Soil Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.85054			
R Square	.72341			
Ad R Square	.72059	Regression 1	1169.58	1169.58
Standard Error	2.13613	Residual 98	447.18	4.56
		F = 256.32	Sign F =	.0000

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	1.78	.111	.850	16.01	.0000
Constant	15.39	.289		53.25	.0000



A. pseudoplatanus L.Number1. Soil Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.60665			
R Square	.36802			
Ad R Square	.35937	Regression 1	37550.35	37550.35
Standard Error	29.72062	Residual 73	64482.02	883.31
		F = 42.51070	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	15.82	2.42	.606	6.520	.0000
Constant	47.19	14.95		3.155	.0000

Index1. Altitude

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.67102			
R Square	.45026			
Ad R Square	.44273	Regression 1	.01953	.01953
Standard Error	.01807	Residual 73	.02385	.00033
		F = 59.791	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Altitude	1.31	1.69	.671	7.732	.0000
Constant	.069	.004		15.86	.0000

2. Soil Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.80949			
R Square	.65527			
Ad R Square	.64569	Regression 2	.02843	.01421
Standard Error	.01441	Residual 72	.01495	.00021
		F = 68.428	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Altitude	4.76	5.44	2.43	8.75	.0000
Soil Dept	-.03	.005	-1.81	-6.54	.0000

Guard1. Altitude

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.49803			
R Square	.24805			
Ad R Square	.23773	Regression 1	441.65	441.65
Standard Error	4.28276	Residual 73	1338.97	18.34
		F = 24.07	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Altitude	-.02	.04	-.498	-4.9	.0000
Constant	23.27	1.04		22.30	.0000

*P. lanceolata* L.Number1. Soil Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.49854			
R Square	.24854			
Ad R Square	.24475	Regression 1	242248.54	242248.55
Standard Error	60.82020	Residual 198	732421.05	3699.09
		F = 65.49	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	-19.73	2.44	-.499	-8.09	.0000
Constant	288.00	8.23		35.01	.0000

2. Soil Moisture

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.58122			
R Square	.33782			
Ad R Square	.33110	Regression 2	329261.60	164630.80
Standard Error	57.23795	Residual 197	645407.99	3276.18
		F = 50.25	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	-28.30	2.83	-.71	-9.99	.0000
Soil Moisture	1.87	.36	.37	5.15	.0000
Constant	1.87	.36	.37	5.15	.0000

Index1. Altitude

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.51180			
R Square	.26194			
Ad R Square	.25821	Regression 1	.06348	.06348
Standard Error	.3006	Residual 198	.17886	.00090
		F = 70.27	Sign F =	.0000

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Altitude	1.19	1.418	.512	8.38	.0000
Constant	.24	.004		55.89	.0000

2. Soil Moisture

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.55870			
R Square	.31215			
Ad R Square	.30516	Regression 2	.07564	.03782
Standard Error	.02909	Residual 197	.16669	.00085
		F = 44.69	Sign F =	.0000

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Altitude	1.29	1.40	.56	9.23	.0000
Soil Moisture	5.79	1.526	.23	3.79	.0002
Constant	.22	.007		31.60	.0000

3. Soil Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.61475			
R Square	.37792			
Ad R Square	.36839	Regression 3	.09158	.03053
Standard Error	.02773	Residual 196	.150758	.00077
		F = 39.69	Sign F =	.0000

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Altitude	1.024	1.46	.44	7.01	.0000
Soil Moisture	.001	1.76	.41	5.85	.0000
Soil Depth	-.007	.002	-.35	-4.55	.0000
Constant	.234	.007		32.45	.0000

Guard1. Depth

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.26604			
R Square	.07078			
Ad R Square	.06609			
Standard Error	3.08323			
		Regression 1	143.37	143.37
		Residual 198	1882.25	9.51
		F = 15.08	Sign F = .0001	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	.48	.123	.26	3.88	.0000
Constant	20.38	.417		48.88	.0000

2. Altitude

		<u>Analysis of Variance</u>		
		<u>DF</u>	<u>Sum of Squares</u>	<u>Mean Square</u>
Multiple R	.32311			
R Square	.10440			
Ad R Square	.09530			
Standard Error	3.03461			
		Regression 2	211.47	105.73
		Residual 197	1814.14	9.21
		F = 11.48	Sign F = .0000	

Variables in Equation

Variable	B	SE B	BETA	T	SIG T
Soil Depth	.641	.135	.355	4.74	.0000
Altitude	.004	.001	.204	2.72	.0071
Constant	18.75	.724		25.90	.0000

### APPENDIX 3

#### CLIMATIC DATA

Climatic data was obtained from Durham University Observatory, Sunderland Polytechnic and Widdybank Fell Weather Station in order to compare the climate of the different regions during the summer.

##### Data from Durham University Observatory (May-July 1991)

<u>Month</u>	<u>Mean Temp</u> (°C)	<u>Total Rainfall</u> (mm)	<u>Relative Humidity</u> (%)	<u>Total Sunshine</u> (hrs)	<u>Mean Wind Speed</u> (KM hr)
May	10.3	19.3	73	124.3	8.5
June	11.3	53.9	75	152.2	9.0
July	16.5	43.9	76	181.6	8.6

##### Widdybank Fell Weather Station Data (May-July 1991)

<u>Month</u>	<u>Mean Temp</u> (°C)	<u>Total Rainfall</u> (mm)	<u>Total Sunshine</u> (hrs)	<u>Mean Wind Speed</u> (KM hr)
May	10.8	24.8	130.7	23.3
June	11.4	128.0	143.3	24.5
July	17.6	61.5	165.5	23.0

##### Sunderland Polytechnic Data (May-July 1991)

<u>Month</u>	<u>Mean Temp</u> (°C)	<u>Total Rainfall</u> (mm)	<u>Total Sunshine</u> (hrs)	<u>Mean Wind Speed</u> (KM hr)
May	9.9	44.6	195.4	17.0
June	13.0	60.2	179.7	15.0
July	15.5	50.0	186.9	14.1

